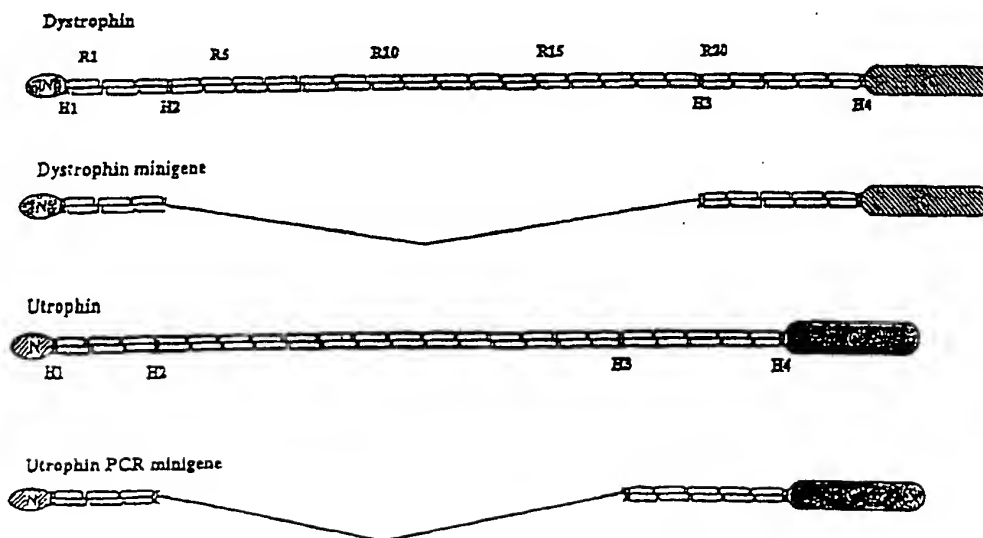




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(71) Applicant (for all designated States except US): MEDICAL RESEARCH COUNCIL [GB/GB]; 20 Park Crescent, London WIN 4AL (GB).			
(72) Inventors; and			
(75) Inventors/Applicants (for US only): TINSLEY, Jonathon, Mark [GB/GB]; Genetics Laboratory, Dept. of Biochemistry, South Parks Road, Oxford OX1 3QU (GB). DAVIES, Kay, Elizabeth [GB/GB]; Genetics Laboratory, Dept. of Biochemistry, South Parks Road, Oxford OX1 3QU (GB).			
(74) Agents: WALTON, Seán, M. et al.; Mewburn Ellis, York House, 23 Kingsway, London WC2B 6HP (GB).			

(54) Title: UTROPHIN GENE EXPRESSION



(57) Abstract

Nucleic acid from which a polypeptide with utrophin function can be expressed, especially mini-genes and chimaeric constructs. Expression significantly decreases the severity of the dystrophic muscle phenotype in an animal model, indicating usefulness in treatment of muscular dystrophy. The nucleic acid and encoded polypeptides are also useful in screening for substances to modulate utrophin binding to actin and/or the dystrophin protein complex.

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UTROPHIN GENE EXPRESSION

The present invention generally relates to the provision of nucleic acid from which a polypeptide with utrophin function can be expressed, especially mini-
5 genes and chimaeric constructs. Expression of a utrophin transgene significantly decreases the severity of the dystrophic muscle phenotype in an animal model.

The severe muscle wasting disorders, Duchenne muscular dystrophy (DMD) and the less debilitating
10 Becker muscular dystrophy (BMD) are due to mutations in the dystrophin gene. Dystrophin is a large cytoskeletal protein which in muscle is located at the cytoplasmic surface of the sarcolemma, the neuromuscular junction (NMJ) and myotendinous junction
15 (MTJ). The protein is composed of four domains: an actin-binding domain (shown in vitro to bind actin), a rod domain containing triple helical repeats, a cysteine rich (CR) domain and a carboxy-terminal (CT) domain. The majority of the CRCT binds to a complex of
20 proteins and glycoproteins (called the dystrophin protein complex, DPC) spanning the sarcolemma. This complex consists of cytoskeletal syntrophins and dystrobrevin, transmembrane, β -dystroglycan, α -, β - δ -, γ -sarcoglycans and extracellular α -dystroglycan. The DPC
25 links to laminin- α 2 (merosin) in the extracellular matrix and to the actin cytoskeleton via dystrophin within the cell. The breakdown of the integrity of the DPC due to the loss of, or impairment of dystrophin

function, leads to muscle degeneration and the DMD phenotype. The structure of dystrophin and protein interactions within the DPC have been recently reviewed [1,2,3].

5 There are various approaches which can be adopted for the gene therapy of DMD. These include myoblast transfer, retroviral infection, adenoviral infection and direct injection of plasmid DNA. In most cases the dystrophin gene used in the experiments generates a
10 truncated protein approximately half the size of the full size protein. This dystrophin minigene was modelled on a natural mutation identified in a very mild Becker patient [4]. The cloned version of this truncated minigene is able to reverse the pathological
15 phenotype in the dystrophin deficient *mdx* mouse [5,6,7] and has had limited success when delivered to *mdx* muscle by viral vectors [8,9,10]. Although some progress is being made in each of these areas using the *mdx* mouse as a model system, there are problems related
20 to the number of muscle cells that can be made dystrophin positive, the levels of expression of the gene and the duration of expression [11]. Another problem to be addressed is the rejection of cells expressing dystrophin because of immunological
25 intolerance i.e. dystrophin within these cells will appear foreign to the host immune system given that most DMD patients will never have expressed dystrophin [12,13].

In order to circumvent some of these problems, possibilities of compensating for dystrophin loss using a related protein, utrophin, are being explored.

Utrophin is a 395kDa protein encoded by a gene
5 located on chromosome 6q24 and shown to have strong
sequence similarity to dystrophin [14]. The actin
binding domain of dystrophin and utrophin has 85%
similarity and the DPC binding region has 88%
similarity. Both of these domains have been shown to
10 function as predicted *in vitro*. The structure and
potential protein interactions are described in detail
in reviews [1,2,3].

There is a substantial body of evidence
demonstrating that utrophin is capable of localising to
15 the sarcolemma. During normal fetal muscle development
there is increased utrophin expression, localised to
the sarcolemma up until 18 weeks and 20 days gestation
in human and mouse respectively. After this time the
utrophin sarcolemmal staining steadily decreases to the
20 significantly lower adult levels shortly before birth
where utrophin is localised almost exclusively to the
NMJ and MTJ [15,16,17]. The decrease in utrophin
expression coincides with increased expression of
dystrophin [17]. Many studies have shown that utrophin
25 is bound to the sarcolemma in DMD and BMD patients.
However the levels of utrophin localised at the
sarcolemma vary from report to report [18,19,20,21].
In some other non Xp21 myopathies, utrophin and

dystrophin are simultaneously bound to the sarcolemma of adult skeletal muscle [22].

High levels of utrophin may protect muscle from the consequences of dystrophin loss. Matsumara *et al.* [23] demonstrated that purified membranes from the *mdx* mouse contained complexes of utrophin and the DPC. When quadricep muscles (which show necrosis) from these mice were analysed by immunoblotting, the level of utrophin remained approximately the same, however the level of the α -dystroglycan was drastically reduced. In cardiac muscle (which shows no pathology) the level of utrophin was elevated four fold with no loss of the α -dystroglycan. Immunocytochemical analysis of other *mdx* small calibre skeletal muscles (extraocular and toe) which also have no pathology shows increased utrophin expression and normal levels of α -sarcoglycan. This result suggests that the increased levels of utrophin interacts with the DPC (or an antigenically related complex) at the sarcolemma and prevents loss of the complex thus the structure of these cells remains normal. In the *mdx* mouse, utrophin levels in muscle remain elevated soon after birth compared with normal mice; however once the utrophin levels have decreased to the adult levels (about 1 week after birth), the first signs of muscle fibre necrosis are detected [15,16].

Thus, in certain circumstances utrophin can localise to the sarcolemma probably at the same binding

sites as dystrophin, namely actin and the DPC. If the expression of utrophin is high enough, it may maintain the DPC and thus alleviate the DMD phenotype. It is unlikely that such external upregulation could be

5 tightly controlled giving rise to potentially high levels of utrophin within the cell. However, this may not be a problem as Cox et al. [24] have demonstrated that gross over expression of dystrophin in the muscle of transgenic *mdx* mice reverts the muscle pathology to

10 normal with no obvious detrimental side effects.

The present invention has arisen from cloning of nucleic acid encoding utrophin and fragments of utrophin from various species. The original aim was to clone nucleic acid encoding human utrophin, but major

15 problems were encountered. A previous paper (14) reported the amino acid sequence of utrophin (so-called "dystrophin-related protein"), obtained by cloning of overlapping cDNAs. However, two regions around the amino terminal actin binding domain were not

20 represented in these clones. These regions could be amplified by PCR and sequenced, but it has proved not to be possible to clone them. Either clones which should have included these regions were rearranged (as determined by restriction mapping) or simply no clones

25 were isolated even if highly recombination deficient *E. coli* host strains (SURE and STBL2) were used. The gaps in the sequence were identified by comparing the sequence generated from the utrophin cDNAs to the

published human dystrophin sequence. It became apparent as further utrophin clones were isolated, none spanned these two gaps.

Sequence information obtained from the amino
5 terminus of the human cDNA was used to design probes and rat and mouse cDNA libraries were screened. Rat cDNAs were also unstable or rearranged in the region corresponding to the unclonable regions in the human sequence. Some large rat clones covering these regions
10 were obtained, but all attempts to generate subclones failed due to rearrangements of the inserts as determined by restriction mapping. Surprisingly, in view of the difficulties with the human and rat sequences, cDNA from the mouse library, covering the
15 regions in question, was found to be stable and amenable to further manipulation including the generation of smaller subclones.

Figure 1 shows a comparison between human, rat and mouse utrophin nucleotide sequences encoding part
20 of the amino-terminal portion of the respective proteins. The unclonable regions of the human gene are underlined.

This cloning work enables for the first time the construction of a nucleic acid molecule from which a
25 polypeptide with utrophin function can be expressed.

Furthermore, by way of analogy with the success achieved with a dystrophin mini-gene (from which a truncated version of dystrophin is expressed) the

present invention provides "utrophin mini-genes" and polypeptides encoded thereby. To overcome the problem of unclonability of regions of the human utrophin gene sequence, the present inventors have realised that it is possible to employ a sequence of nucleotides derived from the mouse utrophin gene in a chimaeric construct to provide for expression of a polypeptide with utrophin function.

According to a first aspect of the present invention there is provided a nucleic acid molecule comprising a sequence of nucleotides encoding a polypeptide with utrophin function.

A polypeptide with utrophin function is able to bind actin and able to bind the dystrophin protein complex (DPC).

Polypeptides with utrophin function are generally distinguishable immunologically from dystrophin polypeptides. For example, they may comprise at least one epitope not found in dystrophin. Polypeptides with utrophin function may be identified using specific polyclonal or monoclonal antibodies which do not cross-react with dystrophin. If a polypeptide is able to bind actin and able to bind the dystrophin protein complex and at least one antibody can bind it which cannot bind dystrophin, then the polypeptide has utrophin function. In a preferred embodiment, the polypeptide can be bound by an antibody which binds utrophin but not dystrophin, in other words the

polypeptide shares at least one epitope with utrophin which epitope is not found in dystrophin. In another embodiment, the polypeptide does not contain an epitope found in dystrophin, such that the polypeptide is not bound by an antibody which binds dystrophin. In such a case, the epitope recognised by the antibody which binds dystrophin may be one not found in utrophin. The polypeptide may contain no epitope found in dystrophin. The immunological comparison may be made with human utrophin and/or dystrophin, especially if the polypeptide with utrophin function is intended for human use, or with the utrophin and/or dystrophin of the species in which use is intended, e.g. mouse. Mouse monoclonal antibodies MANCH07 and MANNUT1[31] were used in the work described herein. Standard in vitro binding assays may be used to assess immunological cross-reactivity of a polypeptide.

Thus, the polypeptide comprises an actin-binding domain and a dystrophin protein complex (DPC)-binding domain and utrophin-like as opposed to dystrophin-like, e.g. as determined immunologically.

Preferably the encoding sequence comprises a human sequence, i.e. a sequence obtainable from the genome of a human cell.

Comparison of various amino acid sequences reveals the following % similarities (calculated using the method of Needleman and Wunsch (1974) *J. Mol. Biol.* 48: 443-453, performed using the GAP program from

the Winsconsin Package v8, Genetics Computer Group, 575
Science Drive, Madison, Wisconsin 53711, USA) and
identities:

- full length human dystrophin v. human utrophin
5 69% similarity, 50.7% identity;
- full length human utrophin v. rat utrophin
 93.2% similarity, 87.1% identity;
- full length human dystrophin v. mouse dystrophin
 95.4% similarity, 91.2% identity;
- 10 - human dystrophin C-terminus v. human utrophin C-
terminus 84.1% similarity, 73.6% identity.

As noted, the present invention is only concerned
with "utrophin-like" molecules, not "dystrophin-like"
molecules. Thus, polypeptides according to the present
15 invention (e.g. as encoded by nucleic acid according to
the invention) may have an amino acid sequence which is
greater than about 75% similar, preferably greater than
about 80%, about 85%, about 90%, about 95% or about 98%
similarity to the amino acid sequence of Figure 3 or
20 the amino acid sequence of Figure 9, taken over the
full length. The polypeptides may have an amino acid
identity of greater than about 55% identity, preferably
greater than about 60% identity, about 70%, about 80%,
about 90%, about 95% or about 98% identity over the
25 full-length. The levels of similarity and/or identity
may be lower outside the C-terminal, DPC-binding domain
provided the DPC-binding domain has greater than about
85% similarity, preferably greater than about 90%,

about 95% or about 98% similarity with the DPC-binding domain amino acid sequence of Figure 3 or Figure 9, or has greater than about 80%, preferably greater than about 85%, about 90%, about 95% or about 98% identity with the DPC-binding domain amino acid sequence of Figure 3 or Figure 9. Particular amino acid sequence variants or derivatives may have a sequence which differs from the sequence of Figure 3 or Figure 9 by one or more of insertion, addition, substitution or deletion of 1 amino acid, 2, 3, 4, 5-10, 10-20 20-30, 30-50, 50-100, 100-150, or more than 150 amino acids.

The nucleic acid molecule may be an isolate, or in an isolated and/or purified form, that is to say not in an environment in which it is found in nature, removed from its natural environment. It may be free from other nucleic acid obtainable from the same species, e.g. encoding another polypeptide.

The nucleic acid molecule may be one which is not found in nature. For example, the sequence of nucleotides may form part of a cloning vector and/or an expression vector, as discussed further below. The sequence of nucleotides may represent a variant or derivative of a naturally occurring sequence by virtue of comprising an addition, insertion, deletion and/or substitution of one or more nucleotides with respect to the natural sequence, provided preferably that the encoded polypeptide has the specified characteristics. The addition, insertion, deletion and/or substitution

of one or more nucleotides may or may not be reflected in an alteration in the encoded amino acid sequence, depending on the genetic code.

Preferably, the nucleic acid molecule is a "mini-gene", i.e. the polypeptide encoded does not correspond to full-length utrophin but is rather shorter, a truncated version. For instance, part or all of the rod domain may be missing, such that the polypeptide comprises an actin-binding domain and a DPC-binding domain but is shorter than naturally occurring utrophin. In a full-length utrophin gene, the actin-binding domain is encoded by nucleotides 1-739, while the DPC-binding domain (CRCT) is encoded by nucleotides 8499-10301 (where 1 represents the start of translation; Figure 2A). The respective domains in the polypeptide encoded by a mini-gene according to the invention may comprise amino acids corresponding to those encoded by these nucleotides in the full-length coding sequence.

Dystrophin mini-genes have been shown to be active in animal models (as discussed). Advantages of a mini-gene over a sequence encoding a full-length utrophin molecule or derivative thereof include easier manipulation and inclusion in vectors, such as adenoviral and retroviral vectors for delivery and expression.

A further preferred non-naturally occurring molecule encoding a polypeptide with the specified

characteristics is a chimaeric construct wherein the encoding sequence comprises a sequence obtainable from one mammal, preferably human ("a human sequence"), and a sequence obtainable from another mammal, preferably mouse ("a mouse sequence"). Such a chimaeric construct may of course comprise the addition, insertion, substitution and/or deletion of one or more nucleotides with respect to the parent mammalian sequences from which it is derived. Preferably, the part of the coding sequence which encodes the actin-binding domain comprises a sequence of nucleotides obtainable from the mouse, or other non-human mammal, or a sequence of nucleotides derived from a sequence obtainable from the mouse, or other non-human mammal.

In a preferred embodiment, the sequence of nucleotides encoding the polypeptide comprises sequence GAGGCAC at residues 331-337 and/or the sequence GATTGTGGATGAAAACAGTGGG at residues 1453-1475 (using the conventional numbering from the initiation codon ATG), and a sequence obtainable from a human.

The nucleic acid molecule may comprise a nucleotide sequence encoding a sequence of amino acids shown in Figure 1. As discussed, the encoding sequence may be chimaeric, i.e. comprise sequences of nucleotides from different species, e.g. a sequence from or derivable from a human and a sequence from or derivable from a mouse or other non-human mammal.

A chimaeric mini-gene encoding sequence according

to the present invention is shown in Figure 3.

Preferred embodiments of the present invention include a nucleic acid molecule comprising a sequence of nucleotides encoding a polypeptide which has an actin-binding domain and a DPC-binding domain and which

5 polypeptide comprises an amino acid sequence encoded by a sequence of nucleotides shown in Figure 3, a nucleic acid molecule comprising a sequence of nucleotides encoding a variant, allele or derivative of such a

10 polypeptide by way of addition, substitution, insertion and/or deletion of one or more amino acids, and a nucleic acid molecule comprising a sequence of nucleotides which is a variant, allele or derivative of the sequence shown in Figure 3, by way of addition,

15 substitution, insertion and/or deletion of one or more nucleotides, with or without a change in the encoded amino acid sequence with respect to the amino acid sequence encoded by a sequence of nucleotides shown in Figure 3. The proviso is that the encoded polypeptide

20 is "utrophin-like" rather than "dystrophin-like", e.g. as determined immunologically as discussed.

One particular variant or derivative of the sequence of Figure 3 has a sequence as shown in Figure 9, which is a "full-length" utrophin construct,

25 including rod domain sequences not included in the mini-gene of Figure 3.

The sequences of Figure 3 and Figure 9 include some positions at which the precise residue is left

open (marked by "N" in the nucleotide sequence and "X" in the amino acid sequence). Comparison of the human, mouse and rat utrophin sequences in this region (Figure 10) shows that the human and rat amino acid sequences are absolutely conserved here. Accordingly, the twelve "X's" in Figures 3 and 9 may represent the amino acid sequence DKKSIIMYLTSL. Instead, in accordance with the discussion of variants and derivatives herein, a polypeptide according to the invention (as encoded by nucleic acid according to the invention) may include a variant or derivative sequence, by way of one or more of insertion, addition, substitution or deletion of one or more amino acids of the sequence DKKSIIMYLTSL, in the position marked by the X's in Figures 3 and 9.

Nucleic acid according to the present invention is obtainable by hybridising nucleic acid of target cells (e.g. human, mouse, rat) with one or more oligo- or poly-nucleotides with sequences designed based on the sequence information presented in Figure 1, Figure 3 or Figure 9. Thus, the full mouse sequence, or the sequence in the region marked by the X's in Figures 3 and 9, may be obtained by probing or PCR using sequence information provided herein (e.g. Figure 1).

Nucleic acid according to the present invention is obtainable using one or more oligonucleotide probes or primers designed to hybridise with one or more fragments of a nucleic acid sequence shown in Figure 1, Figure 3 or Figure 9, particularly fragments of

relatively rare sequence, based on codon usage or statistical analysis. The amino acid sequence information provided may be used in design of degenerate probes/primers or "long" probes. A primer
5 designed to hybridise with a fragment of the nucleic acid sequence shown may be used in conjunction with one or more oligonucleotides designed to hybridise to a sequence in a cloning vector within which target nucleic acid has been cloned, or in so-called "RACE"
10 (rapid amplification of cDNA ends) in which cDNA's in a library are ligated to an oligonucleotide linker and PCR is performed using a primer which hybridises with the sequence shown in the figure and a primer which hybridises to the oligonucleotide linker.

15 Nucleic acid isolated and/or purified from one or more cells (e.g. human, mouse) or a nucleic acid library derived from nucleic acid isolated and/or purified from cells (e.g. a cDNA library derived from mRNA isolated from the cells), may be probed under
20 conditions for selective hybridisation and/or subjected to a specific nucleic acid amplification reaction such as the polymerase chain reaction (PCR).

A method may include hybridisation of one or more (e.g. two) probes or primers to target nucleic acid.
25 Where the nucleic acid is double-stranded DNA, hybridisation will generally be preceded by denaturation to produce single-stranded DNA. The hybridisation may be as part of a PCR procedure, or as

part of a probing procedure not involving PCR. An example procedure would be a combination of PCR and low stringency hybridisation. A screening procedure, chosen from the many available to those skilled in the art, is used to identify successful hybridisation events and isolated hybridised nucleic acid.

Probing may employ the standard Southern blotting technique. For instance DNA may be extracted from cells and digested with different restriction enzymes. Restriction fragments may then be separated by electrophoresis on an agarose gel, before denaturation and transfer to a nitrocellulose filter. Labelled probe may be hybridised to the DNA fragments on the filter and binding determined. DNA for probing may be prepared from RNA preparations from cells.

Preliminary experiments may be performed by hybridising under low stringency conditions various probes to Southern blots of DNA digested with restriction enzymes. Suitable conditions would be achieved when a large number of hybridising fragments were obtained while the background hybridisation was low. Using these conditions nucleic acid libraries, e.g. cDNA libraries representative of expressed sequences, may be searched.

It may be necessary for one or more gene fragments to be ligated to generate a full-length coding sequence. Also, where a full-length encoding nucleic acid molecule has not been obtained, a smaller

molecule representing part of the full molecule, may be used to obtain full-length clones. Inserts may be prepared from partial cDNA clones and used to screen cDNA libraries.

5 Those skilled in the art are well able to employ suitable conditions of the desired stringency for selective hybridisation, taking into account factors such as oligonucleotide length and base composition, temperature and so on.

10 Systems for cloning and expression of a polypeptide in a variety of different host cells are well known. Suitable host cells include bacteria, mammalian cells, yeast and baculovirus systems. Mammalian cell lines available in the art for
15 expression of a heterologous polypeptide include Chinese hamster ovary cells, HeLa cells, baby hamster kidney cells and many others. A common, preferred bacterial host is *E. coli*.

 Nucleic acid according to the present invention
20 may form part of a cloning vector and/or a vector from which the encoded polypeptide may be expressed. Suitable vectors can be chosen or constructed, containing appropriate and appropriately positioned regulatory sequences, including promoter sequences,
25 terminator fragments, polyadenylation sequences, enhancer sequences, marker genes and other sequences as appropriate. Vectors may be plasmids, viral e.g. 'phage, or phagemid, as appropriate. For further

details see, for example, *Molecular Cloning: a Laboratory Manual*: 2nd edition, Sambrook et al., 1989, Cold Spring Harbor Laboratory Press. Many known techniques and protocols for manipulation of nucleic acid, for example in preparation of nucleic acid constructs, mutagenesis, sequencing, introduction of DNA into cells and gene expression, and analysis of proteins, are described in detail in *Short Protocols in Molecular Biology*, Second Edition, Ausubel et al. eds., John Wiley & Sons, 1992. The disclosures of Sambrook et al. and Ausubel et al. are incorporated herein by reference.

Thus, a further aspect of the present invention provides a host cell containing nucleic acid as disclosed herein. A still further aspect provides a method comprising introducing such nucleic acid into a host cell. The introduction may employ any available technique. For eukaryotic cells, suitable techniques may include calcium phosphate transfection, DEAE-Dextran, electroporation, liposome-mediated transfection and transduction using retrovirus or other virus, e.g. vaccinia or, for insect cells, baculovirus. For bacterial cells, suitable techniques may include calcium chloride transformation, electroporation and transfection using bacteriophage.

The introduction may be followed by causing or allowing expression from the nucleic acid, e.g. by culturing host cells under conditions for expression of

the gene.

In one embodiment, the nucleic acid of the invention is integrated into the genome (e.g. chromosome) of the host cell. Integration may be promoted by inclusion of sequences which promote
5 recombination with the genome, in accordance with standard techniques.

The invention also provides a mammal, such as a human, primate or rodent, preferably rat or mouse,
10 comprising a host cell as provided, and methods of production and use of such a mammal. The mammal may be non-human. Transgenic animals, particularly mice, can be generated using any available technique. Particularly suitable for purposes of study are *mdx*
15 mice or others with a dystrophic phenotype.

The polypeptide encoded by the nucleic acid may be expressed from the nucleic acid *in vitro*, e.g. in a cell-free system or in cultured cells, or *in vivo*. *In vitro* expression may be useful in determining ability
20 of the polypeptide to bind to actin and/or DPC. This may be useful in testing or screening for substances able to modulate one or both of these binding activities. In particular, substances able to increase actin and/or DPC binding of the polypeptide will add to
25 the repertoire of molecules available for potential pharmaceutical/therapeutic exploitation. Such substances, identified as modulators of one or both of the binding activities of the polypeptide, following

expression of the polypeptide from encoding nucleic acid therefor, may be investigated further and may be manufactured and/or used in preparation of a medicament, pharmaceutical composition or drug which may subsequently be administered to an individual. In vivo expression is discussed further below.

According to a further aspect of the present invention there is provided a polypeptide with utrophin function (other than utrophin itself). Such a polypeptide comprises an actin-binding domain and a DPC-binding domain and is immunologically recognisable as utrophin-like rather than dystrophin-like, as discussed, not-being a naturally occurring polypeptide. The polypeptide may be any of those discussed above as being encoded by nucleic acid according to the present invention. In particular, the polypeptide may be shorter than naturally occurring full-length utrophin, for example by virtue of lacking all or part of the rod domain. The actin-binding and DPC-binding domains may correspond to those of human, mouse or other non-human utrophin or may be derived therefrom by way of addition, substitution, insertion and/or deletion of one or more amino acids. The polypeptide may be chimaeric, comprising sequences of amino acids from or derived from different species, e.g. human and mouse, as discussed.

A convenient way of producing a polypeptide according to the present invention is to express

nucleic acid encoding it. Accordingly, methods of making such polypeptides by expression from encoding nucleic acid therefor are provided by the present invention, *in vitro*, e.g. in cell-free systems or by
5 culturing host cells under appropriate conditions for expression, or *in vivo*.

Polypeptides and nucleic acid according to the invention may be used in the manufacture of medicaments, compositions, including pharmaceutical
10 formulations, and drugs for delivery to an individual, e.g. a human with muscular dystrophy or a non-human mammal, such as a mouse, as a model for study of the polypeptides, muscular dystrophy and therapy thereof.

For example, a method of treatment practised on
15 the human or animal body in accordance with the present invention may comprise administration to an individual of nucleic acid encoding a polypeptide as disclosed herein. The nucleic acid may form part of a construct enabling expression within cells of the individual.
20 Nucleic acid may be introduced into cells using a retroviral vector, preferably one which will not transform cells, or using liposome technology.

Administration is preferably in a "therapeutically effective amount", this being
25 sufficient to show benefit to a patient. Such benefit may be at least amelioration of at least one symptom. The actual amount administered, and rate and time-course of administration, will depend on the nature and

severity of what is being treated. Prescription of treatment, eg decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors.

- 5 A composition may be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated.

 Pharmaceutical compositions according to the
10 present invention, and for use in accordance with the present invention, may comprise, in addition to active ingredient, a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other materials well known to those skilled in the art. Such materials
15 should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material will depend on the route of administration, which may be oral, or by injection, e.g. cutaneous, subcutaneous or intravenous.

20 Pharmaceutical compositions for oral administration may be in tablet, capsule, powder or liquid form. A tablet may comprise a solid carrier such as gelatin or an adjuvant. Liquid pharmaceutical compositions generally comprise a liquid carrier such
25 as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene

glycol may be included.

For intravenous, cutaneous or subcutaneous injection, or injection at the site of affliction, the active ingredient may be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilisers, buffers, antioxidants and/or other additives may be included, as required.

Injection may be used to deliver nucleic acid to disease sites. Internally, suitable imaging devices may be employed to guide an injecting needle to the desired site.

It may be desirable to remove cells from the body, treat them then return them to the body, or to administer cells derived from cells removed from an individual. This might be appropriate, for example, if muscle stem cells can be isolated. Muscle precursor cells ("mpc") have been used in cell therapy in mdx mice, where implantation of normal mpc gave rise to substantial amounts of dystrophin [25,26,27].

Immunosuppression increases success of cell implantation procedures [13]. Myoblasts may be used to introduce genes into muscle fibres during growth or repair, as has been demonstrated using a replication-

defective retroviral vector to introduce a mini-dystrophin construct into proliferating myogenic cells in tissue culture [28].

Thus, cells in culture may have nucleic acid
5 according to the present invention introduced into them before the cells are grafted into muscles in a patient. Grafting the cells back into the donor has the advantages of a genetically corrected autologous transplant. Nucleic acid may be introduced locally
10 into cells using transfection, electroporation, microinjection, liposomes, lipofecting or as naked DNA or RNA, or using any other suitable technique.

Retroviral vectors have also been used to introduce the dystrophin mini-gene into the myoblasts
15 of spontaneously regenerating muscle of the *mdx* mouse to produce dystrophin-positive fibres [8]. Recombinant replication defective adenoviruses appear particularly effective as an efficient means of introducing constructs into skeletal muscle fibres for persistent
20 expression [29]. See reference 11 for a review of myoblast-based gene therapies.

Adenoviral, retroviral or other viral vectors may be used advantageously for the introduction of a dystrophin sequence according to the present invention
25 into muscle cells. Even though *in vivo* transduction may be restricted to growing or regenerating muscle fibres, retrovirally introduced constructs have the advantage of becoming integrated into the genome of the

host cell, potentially conferring lifelong expression.

Liposomes may be used as vehicles for delivery of nucleic acid constructs to skeletal muscle.

Intravenous injection of constructs in cationic

5 liposomes has resulted in widespread transfection of most tissues, including skeletal muscle [30]. Lack of immunogenicity allows for repeated administration and lack of tissue specificity may be accommodated by choosing a muscle-specific promoter to drive
10 expression.

For use in distinguishing polypeptide with utrophin function from dystrophin and related polypeptides, antibodies may be obtained using techniques which are standard in the art. Methods of
15 producing antibodies include immunising a mammal (eg mouse, rat, rabbit, horse, goat, sheep or monkey) with the protein or a fragment thereof, or a cell or virus which expresses the protein or fragment. Immunisation with DNA encoding a target polypeptide is also possible
20 (see for example Wolff, et al. *Science* 247: 1465-1468 (1990); Tang, et al. *Nature* 356: 152-154 (1992); Ulmer J B, et al. *Science* 259: 1745-1749 (1993)). Antibodies may be obtained from immunised animals using any of a variety of techniques known in the art, and screened,
25 preferably using binding of antibody to antigen of interest. For instance, Western blotting techniques or immunoprecipitation may be used (Armitage et al, 1992, *Nature* 357: 80-82).

The production of monoclonal antibodies is well established in the art. Monoclonal antibodies can be subjected to the techniques of recombinant DNA technology to produce other antibodies or chimeric molecules which retain the specificity of the original antibody. Such techniques may involve introducing DNA encoding the immunoglobulin variable region, or the complementarity determining regions (CDRs), of an antibody to the constant regions, or constant regions plus framework regions, of a different immunoglobulin. See, for instance, EP184187A, GB 2188638A or EP-A-0239400. A hybridoma producing a monoclonal antibody may be subject to genetic mutation or other changes, which may or may not alter the binding specificity of antibodies produced.

As an alternative or supplement to immunising a mammal with a peptide, an antibody specific for a protein may be obtained from a recombinantly produced library of expressed immunoglobulin variable domains, eg using lambda bacteriophage or filamentous bacteriophage which display functional immunoglobulin binding domains on their surfaces; for instance see WO92/01047. The library may be naive, that is constructed from sequences obtained from an organism which has not been immunised with the target, or may be one constructed using sequences obtained from an organism which has been exposed to the antigen of interest (or a fragment thereof).

Antibodies may be modified in a number of ways. Indeed the term "antibody" should be construed as covering any specific binding substance having an binding domain with the required specificity. Thus
5 this covers antibody fragments, derivatives, functional equivalents and homologues of antibodies, including any polypeptide comprising an immunoglobulin binding domain, whether natural or synthetic. Chimaeric molecules comprising an immunoglobulin binding domain,
10 or equivalent, fused to another polypeptide are therefore included. Cloning and expression of chimaeric antibodies are described in EP-A-0120694 and EP-A-0125023.

It has been shown that the function of binding
15 antigens can be performed by fragments of a whole antibody. Example binding fragments are (i) the Fab fragment consisting of VL, VH, CL and CH1 domains; (ii) the Fd fragment consisting of the VH and CH1 domains; (iii) the Fv fragment consisting of the VL and VH
20 domains of a single antibody; (iv) the dAb fragment (Ward, E.S. et al., Nature 341, 544-546 (1989)) which consists of a VH domain; (v) isolated CDR regions; (vi) F(ab')₂ fragments, a bivalent fragment comprising two linked Fab fragments (vii) single chain Fv molecules
25 (scFv), wherein a VH domain and a VL domain are linked by a peptide linker which allows the two domains to associate to form an antigen binding site (Bird et al, Science, 242, 423-426, 1988; Huston et al, PNAS USA,

85, 5879-5883, 1988); (viii) bispecific single chain Fv dimers (PCT/US92/09965) and (ix) "diabodies", multivalent or multispecific fragments constructed by gene fusion (WO94/13804; P. Holliger et al Proc. Natl. Acad. Sci. USA 90 6444-6448, 1993).

Further aspects and embodiments of the present invention, and modifications to aspects and embodiments disclosed herein, will be apparent to those skilled in the art.

10 The following figures are attached hereto:

Figure 1: Figure 1a shows the corresponding parts of nucleotide sequences of the mouse (Moutro), rat (ratutro) and human (humutro) starting from the first amino acid and encompassing the actin binding domain and start of the rod domain. The heavyline represents the unclonable region in rat and human. Figure 1b shows the sequence of the second unclonable region.

Figure 2 is a schematic of the cloning process of PCR6.0. Figure 2A: The bold numbers represent the utrophin transcript in kb and the numbers below the line represent the nucleotide positions of the regions in question where 1 is the start of translation; Figure 2B represents the cDNAs used as template for the PCR; Figure 2C represents the two PCR fragments generated to form PCR6.0.

Figure 3 shows the nucleotide sequence (both

strands) of a "utrophin mini-gene" according to the present invention and whose construction is described herein.

Figure 4 shows a representation of the dystrophin and utrophin polypeptides, showing the various domains, and "mini-genes" comprising only parts of the full-length molecules.

Figure 5: Utrophin transgene construction and expression. A, Scale representation of dystrophin, utrophin and the two truncated transgenes. The repeated spectrin-like repeats (R) and the potential hinge sites (H) are marked. B, Utrophin transgene vector. The N- and C-terminal portions of utrophin were cloned as PCR products using overlapping cDNAs as template. The regions used are indicated by the dotted lines. The PCR product was cloned into a vector containing the 2.2kb human skeletal α -actin (HSA) promoter and regulatory regions ^{20,21} and SV40 large T poly A site. The cloning sites were such that the transgene was located near the beginning of the second HSA untranslated exon and the Asp718/NotI sites were used to liberate the complete fragment. C, Immunoblot of muscle from the utrophin transgenic line F-3 and a non-transgenic C57BL/10 littermate. M, skeletal muscle; H, heart; D, diaphragm.

Figure 6: Decrease in serum CK levels and centralised myofibres in transgenic *mdx* mice. A, Serum creatine kinase levels in male *mdx* mice expressing the

utrophin transgene. Serum creatine kinase levels from 5 week old mice generated from 4 F₃ litters resultant from a male transgenic mouse crossed with female *mdx*. Offspring consisted of male hemizygous *mdx* (M *mdx*),
5 male utrophin transgenic *mdx* (M Tg *mdx*) and heterozygous females (F *mdx*). Female heterozygotes are not significantly different from wild type so can be used as normal controls. The number of mice (n) in each group is shown in parentheses and the mean SE
10 shown by T-bars. B, Proportion of myofibres containing centralised nuclei. The mean SE is shown by T-bars.

Figure 7: Decrease in centralised nuclei in Diaphragm and TA muscle from other truncated utrophin transgenic *mdx* lines (Gerald, George, Grant, Gavin),
15 normal (n) and *mdx* (*mdx*). The mean SE is shown by T-bars.

Figure 8: Decrease in serum creatine kinase from other utrophin transgenic lines, normal (n) and *mdx* (*mdx*). The number of mice in each group is shown in
20 parenthesis.

Figure 9: Full length utrophin coding sequence and encoded amino acid sequence.

Figure 10: Alignment of amino acid sequences for the N-terminal regions of human, mouse and rat
25 utrophin.

All documents cited are incorporated herein by reference.

Example 1*Cloning of utrophin minigenes*

The four cDNAs covering the latter half of the human utrophin transcript were ligated together using overlapping restriction endonuclease sites. The amino-terminal region was reconstructed using the human 92.2 cDNA joined by the common EcoRI restriction site to the stable mouse cDNA clone, JT1. These two constructs were then used as templates for PCR amplification (Figure 2B). Primers were designed to generate two fragments, PCR2.0 and PCR 4.0, containing no untranslated regions which could be ligated in frame to generate a utrophin minigene containing approximately the first 2kb and last 4kb of the utrophin coding sequence (Figure 2C).

The two PCR fragments were ligated together using the HpaI site. The complete DNA sequence of the 6.0kb minigene is shown in Figure 3. The complete 6kb minigene was excised from the vector and ligated into the eukaryotic expression vectors. SV40-pA consists of the SV40 early promoter linked to exon 1 and part of exon 2 (including the intron) of rabbit β globin to facilitate splicing of any cloned insert. This is of particular importance if the construct is to be used to generate transgenic lines. After a single unique blunt restriction site for cloning inserts into, there is the SV40 small T poly A signal sequence. The SV40 promoter

will express the minigene in all tissues. The HSA-pA construct is similar except for the use of the human skeletal α actin promoter and tissue specific regulatory sequences which will direct expression of the minigene product only in skeletal muscle.

Once cloned into the expression vectors the unique HpaI site was used to clone in a PCR generated fragment containing the remainder of the utrophin rod domain. We now have expression vectors containing a truncated and full length utrophin coding sequence.

The unique HpaI restriction site has also been used to clone in a synthetic oligonucleotide coding for the amino acid sequence which is recognised by a specific antibody to the myc protein. This will enable minigene constructs to be localised by virtue of their expression of the myc tag and recognition by the antibody. For utrophin this is a problem as the endogenous gene is expressed in all cell types. The use of the tag will demonstrate the presence of the minigene when delivered in a gene therapy protocol. There are available a number of other tags including the Flag epitope (IBI) and Green Fluorescent Protein (Clontech) which could be used in a similar fashion.

The utrophin minigene generated consists of the same domains and repeats as the dystrophin minigene (Figure 4). The dystrophin minigene was originally copied *in vitro* from a naturally occurring dystrophin mutation which gave rise to a mild Becker muscular

dystrophy phenotype. It has been used successfully in a number of viral vectors being designed for potential gene therapy routes and in transgenic lines which ameliorate the abnormal muscle phenotype in *mdx* mice.

- 5 Thus the utrophin minigene will be suitable for cloning into viral vectors designed for specific tissue expression in potential gene therapy procedures.

Verification of the integrity of minigenes

It was important to screen for the maintenance of an open reading frame in the PCR generated clones given the propensity for *Taq* thermostable polymerase to introduce mutations. The PCR products were cloned into a vector which had RNA polymerase binding sites allowing the cloned insert to be transcribed and translated generating a radiolabelled protein. If expressed proteins were observed of the correct molecular weight it was inferred that the PCR product had no stop mutations. These products were then western blotted to see if they were recognised by utrophin antibodies. A positive result demonstrated that the expressed protein was in the correct frame to generate the epitopes recognised by the antibodies. Ten different clones both for the PCR2.0 and PCR4.0 were screened in this manner. In all cases full length expression was observed. All PCR2.0 and PCR4.0 clones were detected by MANNUT1 [31] (which recognises the actin binding domain) and MANCHO7 [18] (which recognises

the latter half of the carboxy-terminal) respectively.

Two minigenes were constructed from two different PCR2.0 and 4.0 clones which met the criteria above and cloned into the expression vectors. To check the integrity of the completed minigene, COS cells were transiently transfected with both SV40-PCR6.0 minigenes (A4 and B1) and harvested after time points. Expression of the PCR6.0 minigene protein was identified by western blotting using MANNUT1 [31] and MANCHO7 [18].

Similar transfections were done using these constructs then the cells fixed and immunostained using MANCHO7 [18]. Staining of the minigene appeared to be membrane bound suggesting that the actin binding domain or the CRCT or both are functional in order to explain the staining pattern seen.

The myc tag epitope has also been cloned in frame into the unique HpaI site within the minigene. This construct, SV40-PCR6.0-myc, was also transfected into COS cells and immunolocalised using the myc tag mouse monoclonal antibody, 9E10. Again membrane localisation was observed showing that introduction of the 10 amino acids which constitutes the myc tag epitope does not appear to effect the properties of the minigene.

25 Example 2

In Vivo Compensation for Dystrophic Deficiency by

Utrophin Expression

We have tested expressing a utrophin transgene in the dystrophin deficient *mdx* mouse. Our results indicate that high expression of the utrophin transgene in skeletal muscle can reverse the dystrophic pathology. These data suggest that systemic up-regulation of utrophin in DMD patients is a very promising avenue for the development of an effective treatment for this devastating disorder.

10 A truncated utrophin transgene was modeled on the Becker dystrophin transgene which has been shown to correct the dystrophic phenotype of *mdx* mice [5,6] (Fig. 5A). In order to generate high levels of muscle expression the utrophin transgene was driven by the human skeletal alpha actin (HSA) promoter (Fig. 5B). A number of transgenic lines expressing the utrophin transgene were generated with differing levels of transgenic expression. Immunoblot analysis of muscle samples from transgenic lines demonstrating high level expression are shown in Fig. 5C. The multiple fainter bands are probably due to the proteolytic breakdown of the highly expressed transgene product [24]. Line 347 also shows weak expression of the transgene in the heart. Analysis of the F-3 line shows no evidence of transgene expression in heart, brain, kidney, lung, liver, intestine, skin or pancreas was observed. To demonstrate that the utrophin transgene localised to the sarcolemma, immunofluorescence of skeletal muscle

sections was performed using utrophin and dystrophin specific antibodies. Examination of the sarcolemmal localisation pattern of dystrophin and the utrophin transgene in consecutive muscle sections demonstrated that they are able to co-localise *in vivo*. The normal localisation of utrophin in adult skeletal muscle is exclusively at the neuromuscular and myotendenous junctions and in the capillaries and nerves [3,31]. Immunostaining of unfixed 8 μ m TA muscle cryosections was done with 1/25 dilution of G3 (anti-utrophin) or 1/400 dilution of P6 (anti-dystrophin [33]). Initially the sections were blocked in 10% heat inactivated foetal calf serum in 50mM Tris, 150mM NaCl pH7.5 (TBS), then the primary antibody diluted in TBS added and incubated for 1h at room temperature. The slides were washed 4x in TBS for 5min each then incubated for a further hour at room temperature with 1/1000 dilution FITC conjugated sheep anti-rabbit IgG (Sigma) diluted in TBS. Finally the slides were washed as before, mounted with VectaShield (Vector Labs) and photographed using a Leica DMRBE microscope and photomicrograph system.

Although the dystrophin deficient *mdx* mouse is only mildly affected, histological and physiological analysis reveals a number of muscle defects in common with DMD patients including muscle fibre degeneration giving rise to a dramatic elevation of serum creatine kinase (CK) and evidence of massive myofibre

regeneration with most fibres having centrally located nuclei [34]. Thus changes in the levels of serum CK and numbers of centralised nuclei have been used to monitor the pathology of the muscle in a number of transgenic

5 lines expressing dystrophin transgenes in *mdx* mice [5,6,7,24]. Male transgenic F-3 mice carrying the utrophin transgene were crossed with dystrophin deficient female *mdx* mice and the resultant offspring analysed (Fig. 6A). The CK levels of 5 week old male

10 transgenic *mdx* mice had fallen to approximately a quarter of the non transgenic *mdx* male littermates. Females whether transgenic or not have essentially normal levels of serum CK. The reduction in the serum levels of CK in the transgenic male *mdx* littermates

15 signifies a change in the muscle pathology of these mice and implies that a significant decrease in muscle degeneration has occurred. Fig. 6B shows the contrast in numbers of centralised nuclei in frozen sections from the soleus and tibialis anterior (TA) muscle of

20 transgenic and non-transgenic male *mdx* mice. The numbers of centrally nucleated myofibres is markedly reduced in the two muscle types examined showing that the amount of fibre regeneration is decreased. The difference in numbers of central nuclei between the

25 transgenic *mdx* TA (~10%) and soleus (~30%) is probably explained by the fact that the HSA promoter is expressed at lower levels in the slow twitch fibres which essentially populate the soleus muscle compared

to the fast twitch fibres of the TA. This is an important observation as it implies that the levels of utrophin transgene are important for amelioration of the muscle phenotype.

5 Dystrophin is normally associated with a large oligomeric protein complex (dystrophin protein complex; DPC) embedded in the sarcolemma [3,35]. Loss of dystrophin in DMD patients and *mdx* mice also results in a dramatic loss of sarcolemmal DPC [36]. In transgenic
10 *mdx* mice expressing the full length and truncated dystrophin transgenes, re-establishment of components of the DPC at the sarcolemma is an important marker for the restoration of muscle strength by dystrophin transgenes [5,6,7,24]. We looked at the results of
15 immunostaining for components of the DPC in TA muscle from male *mdx* or *mdx* expressing the utrophin transgene. This was as described above. The primary antibodies were goat polyclonal sera to α/β -dystroglycan [37] (FP-B, 1/10), rabbit polyclonal sera to α -sarcoglycan [38]
20 (1/5) and sheep polyclonal sera to γ -sarcoglycan [39] (1/10). FITC conjugated secondary antibodies to goat, rabbit and sheep were diluted 1/50, 1/200 and 1/50 respectively. Sarcolemmal staining of all myofibres by utrophin specific antibody was seen in transgenic
25 muscle. However in the non-transgenic mice there is virtually no sarcolemmal staining apart from neuromuscular junctions and regions likely to contain regenerating fibres. In all cases using polyclonal

antibodies specific to α -sarcoglycan , γ -sarcoglycan and α/β -dystroglycan there was a notable increase in the staining at the sarcolemma of transgenic TA muscle indicating an elevation in correctly localised, sarcolemmal bound DPCs. The increase in sarcolemmal staining of these components in the soleus muscle is greater than the non-transgenic *mdx* males but not as elevated as in the TA. This result suggests that increased utrophin transgene expression correlates with an increase in sarcolemmal bound DPC.

Analysis of the *mdx* diaphragm has shown that this muscle exhibits a continued pattern of degeneration, fibrosis and functional deficit throughout the lifespan of the *mdx* mouse which is comparable to DMD skeletal muscle [40]. Thus for utrophin to be capable of replacing dystrophin, over-expression of utrophin in this muscle has to alter the pathology in a similar way as demonstrated for the dystrophin transgenic *mdx* mice [5,6,7,24]. Immunostaining of diaphragm sections using a utrophin antibody demonstrates the sarcolemmal localisation of the utrophin transgene expressed in the transgenic *mdx* mouse (utro-tg *mdx*) compared to the normal and *mdx* seen is the re-establishment of α -sarcoglycan at the sarcolemma of the transgenic *mdx* diaphragm at levels similar to the normal diaphragm. Sarcolemmal staining of the transgenic *mdx* diaphragm similar to normal is also seen using antibodies specific to α/β -dystroglycan and γ -sarcoglycan (data

not shown). Thus, as in skeletal muscle, expression of the utrophin transgene in diaphragm relocalises the DPC to the sarcolemma. Histological analysis of haematoxylin and eosin stained sections of *mdx* diaphragm shows extensive regions of fibrosis, cellular infiltration and variable myofibre size containing centralised nuclei. However the utrophin transgenic diaphragm looks essentially the same as normal, with no necrosis, regular myofibre size and virtually no centralised nuclei. In the *mdx* diaphragm, even in regions which have no necrosis so appear more histologically normal, on higher magnification the myofibres are still of variable size often containing centralised nuclei which is indicative of continual regeneration. In the utrophin transgenic diaphragm even at higher magnification, the whole muscle appears normal. A return to normal histology and establishment of the DPC are two important observations, as seen with the dystrophin transgenic mice [5,6,7,24], which predicts a major recovery of the utrophin transgenic diaphragm from a dystrophic phenotype.

We have demonstrated a significant decrease in the dystrophic muscle phenotype of *mdx* mice by expressing a utrophin transgene at high levels in the skeletal muscle and the diaphragm. These results, for the first time, strongly suggest that utrophin can replace dystrophin in vivo. This implies that use of small molecules which increase the normal utrophin

muscle expression to compensate and therefore alleviate the consequences of a lack of dystrophin is a promising avenue for DMD therapy. This approach would potentially target all muscles and thus prolong life by conserving the respiratory and cardiac muscles.

Utrophin is expressed in many tissues so a generalised upregulation may not have detrimental side effects. In our experimental animal model, the normal mice expressing the utrophin transgene at high levels appear to suffer no deleterious side effects in their skeletal and diaphragm muscles. A precedent for such a gene therapy approach using butyrate to upregulate fetal haemoglobin is having success in clinical trials of sickle cell disease [41,42]. Only 20-30% of the wild type levels of dystrophin are required to significantly reduce the dystrophic phenotype in *mdx* mice^{9,10}. It will be interesting to determine whether similar levels of utrophin will be adequate to compensate for dystrophin loss. In addition, since utrophin is normally expressed in all tissues, including muscle, the use of this utrophin transgene rather than a dystrophin transgene in conventional gene therapy approaches e.g. using viruses or liposomes may avert any potential immunological responses against the transgene.

Muscle from transgenic *mdx* and *mdx* mice were stressed in vitro. Essentially the test monitors the high mechanical stress produced by force lengthening

during active contraction and measures the decrease in force. The method is essentially described in detail by Deconinck et al [46]. The measure of deterioration is a decrease in the force a muscle can apply. This
5 force drop is irreversible and correlates with the number of damaged muscle fibres. *Mdx* muscle is particularly sensitive to this test and deteriorates greatly [46].

Our data demonstrate that the force drop in *mdx*
10 mice is -55%. However, in the utrophin transgenic littermates the force drop was only -20%. Normal mouse muscle usually has a force drop of -15%. Thus the expression of the utrophin transgene in *mdx* mice considerably decreases the damage caused by large
15 mechanical stress.

Methods

Transgene construction and microinjection

The amino- and carboxy-terminal portions of utrophin were cloned as PCR products using overlapping
20 cDNAs as template then ligated together in-frame to produce the truncated utrophin cDNA. The PCR product was then cloned into a vector containing the 2.2kb human skeletal α -actin (HSA) promoter and regulatory regions [43,44] and SV40 large T poly A site. The
25 cloning sites were such that the transgene was located near the beginning of the second HSA untranslated exon

and the Asp718/NotI sites were used to liberate the complete fragment. Transgenic mice were generated by microinjection of the purified HSA transgene insert into the pronucleus of F₂ hybrid oocytes from
5 C57BL/6xCBA/CA parents [45]. Positive transgenic mice were identified by southern blotting using a probe to the central part of the utrophin transgene. A number of founder F₀ males were bred to generate more offspring for analysis and breeding.

10 Protein analysis

Total muscle extracts were prepared by homogenisation in 1ml extraction buffer (75mM Tris pH6.8, 3.8% SDS, 4M Urea, 20% Glycerol, 5% β -mercaptoethanol) then heated 95°C for 5min. Usually
15 50 μ g of total protein (quantitated using Biorad DC protein assay kit) was loaded onto 6% polyacrylamide gels and transferred to nitrocellulose. Utrophin transgene expression was detected using a 1/200 dilution of mouse anti-utrophin monoclonal antibody
20 (MANCHO7 [18]) and visualised using anti-mouse IgG-POD and chemiluminescence (Boehringer). For sectioning, skeletal muscle samples were removed and immersed in OCT compound (BDH) and frozen in liquid nitrogen cooled isopentane. Diaphragm was removed, cut in half then
25 rolled longitudinally and sandwiched between Ox liver to facilitate orientation and easier sectioning. The sandwich was then frozen. Immunostaining of unfixed

8 μ m cryosections was performed by blocking the sections in 10% heat inactivated foetal calf serum in 50mM Tris, 150mM NaCl pH7.5 (TBS), then the primary antibody diluted in TBS added and incubated for 1h at room temperature. The slides were washed 4x in TBS for 5min each then incubated for a further hour at room temperature with conjugated second antibody diluted in TBS. Finally the slides were washed as before, mounted with VectaShield (Vector Labs) and photographed using a Leica DMRBE microscope and photomicrograph system.

Antibodies used for immunofluorescence

Antibodies were used at the following dilutions. Polyclonal rabbit against utrophin (G3, 1/25), dystrophin (P6 ^[33], 1/400), β 1-syntrophin (syn35, 1/50) α -sarcoglycan ^[38] (1/5). Goat polyclonal against α/β -dystroglycan (FP-B ^[37], 1/10). FITC conjugated secondary antibody to goat (Sigma) and Cy3 conjugated secondary antibody to rabbit (Jackson Laboratories) were diluted 1/50 and 1/200 respectively.

20 *Creatine kinase assay*

Serum CK levels from 4-5 week old mice generated from 4 F₃ litters resultant from a male transgenic mouse crossed with female mdx were assayed. The tail tips were cut off and DNA prepared for Southern blotting to establish the transgenic status of each mouse. Blood was collected simultaneously, allowed to

clot and serum removed. Serum creatine kinase levels were measured using the Boehringer NAC-CK kit and 5 μ l of serum. The rate per minute was averaged over 4 min and calculated as U/l.

5 Example 3

To see if expression of utrophin is beneficial to muscle in the process of regenerating, the myc tagged truncated utrophin minigene under the control of the HSA promoter (HSA-PCR6.0-myc) was directly injected
10 into *mdx* muscle.

Our data demonstrate the sarcolemmal localisation of the utrophin minigene in a proportion of fibres close to the injection site. The utrophin minigene was detected using the antibody 9E10 which is specific to
15 the myc tag epitope. Importantly where 9E10 was localised there was a significant staining of α - and γ -sarcoglycan. The α -sarcoglycan staining was essentially negative in other fibres.

Re-establishment of the dystrophin protein
20 complex has been shown to be an important marker for muscle recovery [5,6,7,24]. This result suggests that even when the disease process has manifested itself, namely the degeneration and regeneration seen in *mdx* muscle, expression of utrophin is beneficial. This is
25 important when considering that in DMD one third of effected boys are new mutations. Thus only when the

first symptoms of DMD manifest themselves after a couple of years after birth can diagnosis be attained.

Example 4

Utilising a PCR strategy to generate fragments
5 from human 1st strand DNA, the remainder of the human
utrophin sequences missing from PCR6.0 were cloned.
The fragment utilised primers which allowed the rod
domain to be cloned into the unique HpaI restriction
site (see Figure 2c) to produce a clone which contained
10 all of the amino acid coding sequence to produce the
complete utrophin protein. Figure 9 shows the DNA
sequence of the full length utrophin construct with the
amino acid sequence shown above using the standard
single letter code.

15 The utrophin full length construct has been
cloned into the human skeletal alpha actin promoter
(HSA) expression construct in a similar manner to that
shown in Figure 5b. This full length utrophin
expression construct has been used to generate
20 transgenic mice capable of expressing the full length
utrophin protein in mouse muscle. Similar experiments
may be performed as described in Example 2 to identify
any differences in the effectiveness of the full length
utrophin protein compared with the truncated utrophin
25 protein in alleviating the muscle pathology in *mdx*
mice.

In order to assess whether high levels of utrophin expression in all tissues is detrimental, to assist in planning therapeutic protocols and in particular choosing between tissue-specific expression or non-specific expression, a mouse model is being developed using the full length utrophin construct. Transgenic mice will be created expressing the full length utrophin protein under the regulation of a promoter which is expressed in all tissues. The promoter chosen for the first experiments is the human Ubiquitin-C promoter which has been shown to express in all tissues. Once these mice are shown to be expressing the full length utrophin transgene they will be monitored to identify any potential side effects caused by abnormally high levels of utrophin.

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CLAIMS

1. A nucleic acid isolate having a sequence of nucleotides encoding a polypeptide including the amino acid sequence of Figure 3.

5

2. Nucleic acid according to claim 1 wherein said sequence of nucleotides is the coding nucleotide sequence of Figure 3.

10 3. Nucleic acid according to claim 1 wherein said sequence of nucleotides is a variant or derivative, by way of one or more of addition, substitution, insertion and deletion of one or more nucleotides, of the coding nucleotide sequence of Figure 3.

15

4. A nucleic acid isolate having a sequence of nucleotides encoding a polypeptide which is able to bind actin and able to bind the dystrophin protein complex, which includes an amino acid sequence which is
20 a variant or derivative, by way of one or more of addition, substitution, insertion and deletion or one or more amino acids, of the amino acid sequence of Figure 3, and which is distinguishable immunologically from dystrophin.

25

5. Nucleic acid according to claim 4 wherein the polypeptide includes the amino acid sequence of Figure

9.

6. Nucleic acid according to claim 5 having the coding nucleotide sequence of Figure 9.

5

7. Nucleic acid according to claim 5 having a coding nucleotide sequence which is a variant or derivative, by one or more of addition, deletion, insertion or substitution of one or more nucleotides, of the coding sequence of Figure 9.

10

8. Nucleic acid according to any preceding claim comprised in a vector.

15 9. Nucleic acid according to claim 8 wherein said vector is an expression vector.

10. A composition including nucleic acid according to any of claims 1 to 9 and a pharmaceutically acceptable excipient.

20

11. A cell containing nucleic acid according to any of claims 1 to 9.

25 12. A cell according to claim 11 which is a muscle cell.

13. A cell according to claim 11 or claim 12 wherein said polypeptide is expressed.

14. A cell according to any of claims 11 to 13 which
5 is in a mammal.

15. A mammal having a cell according to any of claims 11 to 13.

10 16. A mammal containing nucleic acid according to any of claims 1 to 9.

17. A method including introduction of nucleic acid according to any of claims 1 to 9 into a cell.

15

18. A method according to claim 17 wherein said cell is a muscle cell.

19. A method according to claim 17 or claim 18 wherein
20 said introduction takes place in vitro.

20. A method which includes causing or allowing expression of the coding nucleotide sequence of nucleic acid according to any of claims 1 to 9 in a cell.

25

21. A method according to claim 20 wherein the cell is part of a mammal.

22. A method according to claim 20 wherein the expression product is purified and/or isolated following expression.

5 23. A method according to claim 22 wherein the expression product is formulated into a composition which includes at least one additional component, following purification and/or isolation of the expression product.

10

24. A polypeptide as encoded by nucleic acid according to any of claims 1 to 3.

25. A polypeptide as encoded by nucleic acid according
15 to claim 4, excluding natural utrophin.

26. A composition including a polypeptide according to claim 24 or claim 25 and a pharmaceutically acceptable excipient.

20

27. A method for ameliorating one or more symptoms of a dystrophic phenotype in a mammal, the method including providing cells of the mammal with a polypeptide according to claim 24.

25

28. A method for ameliorating one or more symptoms of a dystrophic phenotype in a mammal, the method

including providing cells of the mammal with a polypeptide according to claim 25.

29. A method according to claim 27 or claim 28 wherein
5 the polypeptide is provided to the cells by expression
from encoding nucleic acid administered to the mammal.

30. Use of nucleic acid according to any of claims 1
to 9 in the manufacture of a medicament for treating a
10 dystrophin phenotype in a mammal.

[illegible][illegible][illegible]

297	A	C	A	C	A	T	G	C	A	A	C	C	A	G	T	C	A	G	G	T	A
298	A	C	A	T	C	C	A	A	A	C	T	G	C	A	A	C	C	A	A	C	C
299	A	C	A	T	C	C	A	A	A	C	T	G	C	A	A	C	C	A	A	C	C
300	A	C	A	T	C	C	A	A	A	C	T	G	C	A	A	C	C	A	A	C	C
301	A	C	A	T	C	C	A	A	A	C	T	G	C	A	A	C	C	A	A	C	C

[illegible][illegible]

322 323 324 325 326

322	323	324	325	326
1	2	3	4	5
6	7	8	9	10
11	12	13	14	15
16	17	18	19	20
21	22	23	24	25
26	27	28	29	30
31	32	33	34	35
36	37	38	39	40
41	42	43	44	45
46	47	48	49	50
51	52	53	54	55
56	57	58	59	60
61	62	63	64	65
66	67	68	69	70
71	72	73	74	75
76	77	78	79	80
81	82	83	84	85
86	87	88	89	90
91	92	93	94	95
96	97	98	99	100

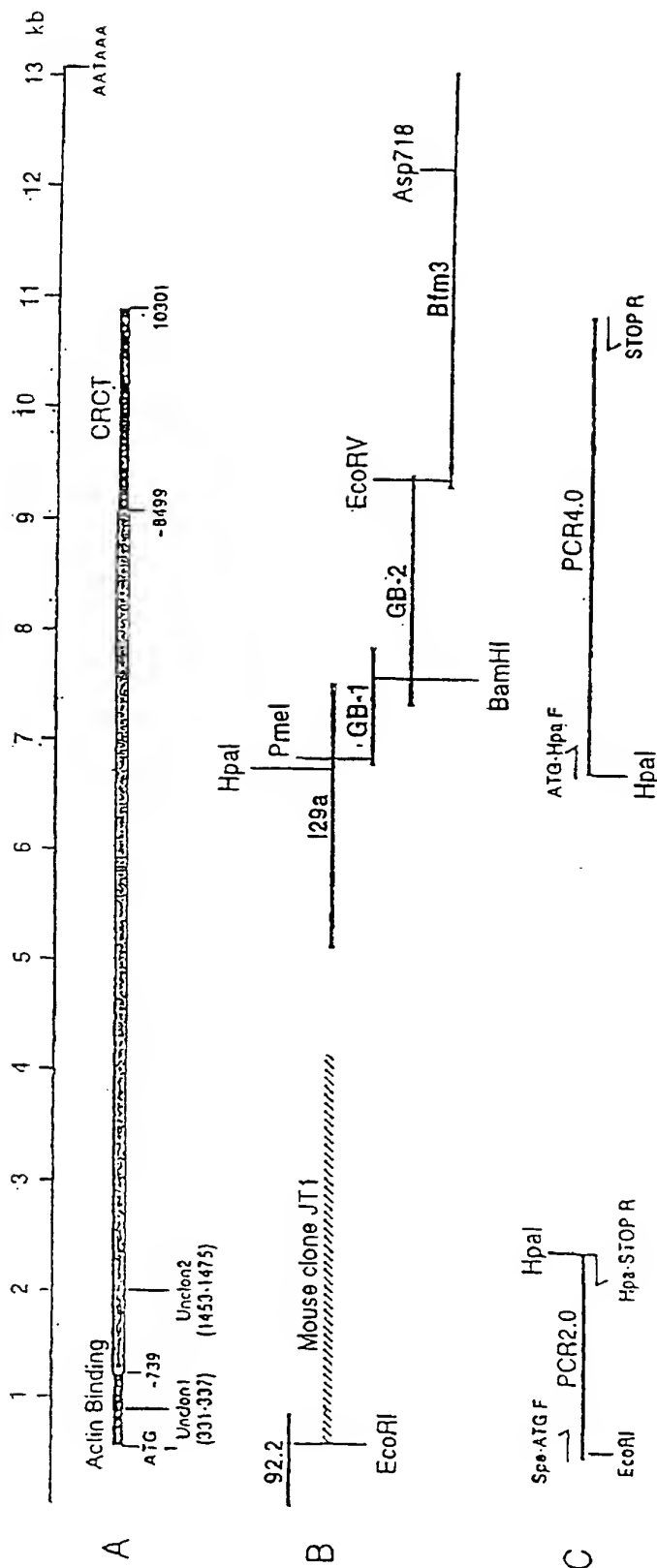
[illegible][illegible][illegible]

Figure 1b
Sequence of 2nd unclonable region in human

Moutro-Unclon2	75	T G A T G A C C T T G C C C T C C C T C C A A A A G C T G C T T G A A A A C A T A A A A A T T T G C A A A A T T G A A G C T G A A C A G G T
Ranuro-Unclon2	75	T G A T G A C C T A C C C T C C C T C C A A A A G C T G C T T G A A A A C A T A A A A A T T T G C A A A A T T G A A G C T G A A C A G G T
Humuro-Unclon2	75	T G A T G A C C T A C C C T C C C T C C A A A A G C T G C T T G A A A A C A T A A A A A T T T G C A A A A T T G A A G C T G A A C A G G T
Moutro-Unclon2	150	G A A G G T A A A T T C C T T A C C T C A C A T G G T G G T G A T T G T G G A T G A A A C A G T G G T G G T G G T G G T G G T G G T G G T
Ranuro-Unclon2	150	G A A G G T A A A T T C C T T A C C T C A C A T G G T G G T G A T T G T G G A T G A A A C A G T G G T G G T G G T G G T G G T G G T
Humuro-Unclon2	150	G A A G G T A A A T T C C T T A C C T C A C A T G G T G G T G A T T G T G G A T G A A A C A G T G G T G G T G G T G G T G G T G G T
Moutro-Unclon2	200	A G A T C A G T T A C A G A A A C T G G T G A G C C C T G G A C A G C T G T A T G C C C C T G G A
Ranuro-Unclon2	200	A G A T C A G T T A C A G A A A C T G G T G A G C C C T G G A C A G C T G T A T G C C C C T G G A
Humuro-Unclon2	200	A G A T C A G T T A C A G A A A C T G G T G A G C C C T G G A C A G C T G T A T G C C C C T G G A

Figure 2

Figure 2



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Figure 3

M A K Y G E H E A S P D N G Q N E
 1 ACTAGTCAAGATGGCCAAGTATGGAGAACATGAAGCCAGTCCCTGACAATGGGCAGAACGA 60
 F S D I I E S R S D E H N D V Q K K T F
 61 ATTCACTGACATCATTGAGTCCAGATCTGATGAACACAATGATGTACAGAAGAAAACCTT 120
 T K W I N A R F S K S G K P P I S D M F
 121 TACCAAATGGATAAACGCTCGATTTTCCAAGAGTGGGAAACCACCCATCAGTGATATGTT 180
 S D L K D G R K L L D L L E G L T G T S
 181 CTCAGACCTCAAAGATGGGAGAAAGCTCTTGGATCTTCTCGAAGGCCTCACAGGAACATC 240
 L P K E R G S T R V H A L N N V N R V L
 241 ATTGCCAAAGGAACGTGGTTCCACAAGGGTGCATGCCTTAAACAATGTCAACCGAGTGCT 300
 Q V L H Q N N V D L V N I G G T D I V D
 301 ACAGGTTTTACATCAGAACAATGTGGACTTGGTGAATATTGGAGGCACGGACATTGTGGA 360
 G N P K L T L G L L W S I I L H W Q V K
 361 TGGAAATCCCAAGCTGACTTTAGGGTTACTCTGGAGCATCATTCTGCACTGGCAGGTGAA 420
 D V M K D I M S D L Q Q T N S E K I L L
 421 GGTGTGCATGAAGATATCATGTGACAGCTGCAGCAGACAAACAGCGAGAAGATCCTGCT 480
 S W V R Q T T R P Y S Q V N V L N F T T
 481 GAGCTGGGTGCGGCAGACCACCAGGCCCTACAGTCAAGTCAACGTCCTCAACTTACCAC 540
 S W T D G L A F N A V L H R H K P D L F
 541 CAGCTGGACCGATGGACTCGCGTTCAACGCCGTGCTCCACCGGCACAAACCAGATCTCTT 600
 S W D R V V K M S P I E R L E H A F S K
 601 CAGCTGGGACAGACTGGTCAAATGTCCCCAATTGAGAGACTTGAACATGCTTTTAGCAA 660
 A H T Y L G I E K L L D P E D V A V H L
 661 GGCCACACTTATTTGGGAATTGAAAAGCTTCTAGATCCTGAAGATGTTGCTGTGCATCT 720
 P X X X X X X X X X X X V E V L P Q Q
 721 CCCNNCCGTTGAGGTGCTTCTCAGCA 780
 V T I D A I R E V E T L P R K Y K K E C
 781 AGTCACGATAGATGCCATCCGAGAGGTGGAGACTCTCCCAAGGAAGTATAAGAAAGAAATG 840
 E E E E I H I Q S A V L A E E G Q S P R
 841 TGAAGAGGAAGAAATTCATATCCAGAGTGCAGTGTGGCAGAGGAAGGCCAGAGTCCCCG 900
 A E T P S T V T E V D M D L D S Y Q I A
 901 AGCTGAGACCCCTAGCACCGTCACTGAAGTGGACATGGATTTGGACAGCTACCAGATAGC 960
 L E E V L T W L L S A E D T F Q E Q D D
 961 GCTAGAGGAAGTGTGACGTGGCTGCTGTCCGCGGAGGACACGTTCCAGGAGCAAGATGA 1020
 I S D D V E E V K E Q F A T H E T F M M
 1021 CATTTCTGATGATGTGGAAGAAGTCAAGAGCAGTTTGCTACCCATGAACTTTTATGAT 1080
 E L T A H Q S S V G S V L Q A G N Q L M
 1081 GGAGCTGACAGCACACCAGAGCAGCGTGGGGAGCGTCTGCAGGCTGGCAACCAGCTGAT 1140
 T Q G T L S E E E E F E I Q E Q M T L L
 1141 GACACAAGGGACTCTGTGTCAGAGGAGGAGGAGTTTGAGATCCAGGAACAGATGACCTTGCT 1200

Figure 3 Continued

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N A R W E A L R V E S M E R Q S R L H D
 1201 GAATGCAAGGTGGGAGGCGCTCCGGGTGGAGAGCATGGAGAGGCAGTCCCGGCTGCACGA 1260
 A L M E L Q K K Q L Q Q L S S W L A L T
 1261 CGCTCTGATGGAGCTGCAGAAGAAACAGCTGCAGCAGCTCTCAAGCTGGCTGGCCCTCAC 1320
 E E R I Q K M E S P P L G D D L P S L Q
 1321 AGAAGAGCGCATTTCAGAAGATGGAGAGCCCTCCGCTGGGTGATGACCTGCCCTCCCTGCA 1380
 K L L Q E H K S L Q N D L E A E Q V K V
 1381 GAAGCTGCTTCAAGAACATAAAAGTTTGCAAAATGACCTTGAAGCTGAACAGGTGAAGGT 1440
 N S L T H M V V I V D E N S G E S A T A
 1441 AAATTCCTTAACTCACATGGTGGTGATTGTGGATGAAAACAGTGGGGAGAGTGCCACAGC 1500
 L L E D Q L Q K L G E R W T A V C R W T
 1501 TCCTTCTGGAAGATCAGTTACAGAACTGGGTGAGCGCTGGACAGCTGTATGCCGCTGGAC 1560
 E E R W N R L Q E I S I L W Q E L L E E
 1561 TGAAGAACGTTGGAACAGGTTGCAAGAAATCAGTATTCTGTGGCAGGAATTATTGGAAGA 1620
 Q C L L E A W L T E K E E A L N K V Q T
 1621 GCAGTGCTCTGTTGGAGGCTTGGCTCACCAGAAAAGGAAGAGGCTTTGAATAAAGTTCAAAC 1680
 S N F K D Q K E L S V S V R R L A I L K
 1681 CAGCAACTTTAAAGACCAGAAGGAACCTAAGTGTGAGTGTCCGGCGTCTGGCTATATTGAA 1740
 E D M E M K R Q T L D Q L S E I G Q D V
 1741 GGAAGACATGGAAATGAAGAGGCAGACTCTGGATCAACTGAGTGAGATTGGCCAGGATGT 1800
 G Q L L S N P K A S K K M N S D S E E L
 1801 GGGCCAATTACTCAGTAATCCCAAGGCATCTAAGAAGATGAACAGTGACTCTGAGGAGCT 1860
 T Q R W D S L V Q R L E D S S N Q V T Q
 1861 AACACAGAGATGGGATTCTCTGGTTCAGAGACTCGAAGACTCTTCTAACCAGGTGACTCA 1920
 A V A K L G M S Q I P Q K D L L E T V H
 1921 GCGGTAGCGAAGCTCGGCATGTCCCAGATTCCACAGAAGGACCTATTGGAGACCGTTCA 1980
 V R E K G M V K K P K Q E L P P P L T K
 1981 TGTGAGAGAAAAAGGATGGTGAAGAAGCCCAAGCAGGAAGTGCCTCCTCCGTTAACAAA 2040
 A E H A M Q K R S T T E L G E N L Q E L
 2041 GGCTGAGCATGCTATGCAAAAGAGATCAACCACCGAATTGGGAGAAAACCTGCAAGAATT 2100
 R D L T Q E M E V H A E K L K W L N R T
 2101 AAGAGACTTAACTCAAGAAATGGAAGTACATGTGAAAAACTCAAATGGCTGAATAGAAC 2160
 E L E M L S D K S L S L P E R D K I S E
 2161 TGAATTGGAGATGCTTTTCAGATAAAAGTCTGAGTTTACCTGAAAGGGATAAAATTTTCAGA 2220
 S L R T V N M T W N K I C R E V P T T L
 2221 AAGCTTAAGGACTGTAAATATGACATGGAATAAGATTTCAGAGAGGTGCCTACCACCCT 2280
 K E C I Q E P S S V S Q T R I A A H P N
 2281 GAAGGAATGCATCCAGGAGCCCAGTTCTGTTTCACAGACAAGGATTGCTGCTCATCTAA 2340
 V Q K V V L V S S A S D I P V Q S H R T
 2341 TGTCCAAAAGGTGGTGTAGTATCATCTGCGTCAGATATTCTGTTTCAGTCTCATCGTAC 2400
 S E I S I P A D L D K T I T E L A D W L
 2401 TTCGGAATTTCAATTCTGCTGATCTTGATAAAACTATAACAGAAGTACCGGACTGGCT 2460

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Figure 3 Continued

V L I D Q M L K S N I V T V G D V E E I
 2461 GGTATTAATCGACCAGATGCTGAAGTCCAACATTGTCACTGTTGGGGATGTAGAAGAGAT 2520
 N K T V S R M K I T K A D L E Q R H P Q
 2521 CAATAAGACCGTTTCCCGAATGAAAATTACAAAGGCTGACTTAGAACAGCGCCATCCTCA 2580
 L D Y V F T L A Q N L K N K A S S S D M
 2581 GCTGGATTATGTTTTTACATTGGCACAGAATTTGAAAAATAAAGCTTCCAGTTCAGATAT 2640
 R T A I T E K L E R V K N Q W D G T Q H
 2641 GAGAACAGCAATTACAGAAAAATTGGAAAGGGTCAAGAACCAGTGGGATGGCACCCAGCA 2700
 G V E L R Q Q Q L E D M I I D S L Q W D
 2701 TGGCGTTGAGCTAAGACAGCAGCAGCTTGAGGACATGATTATTGACAGTCTTCAGTGGGA 2760
 D H R E E T E E L M R K Y E A R L Y I L
 2761 TGACCATAGGGAGGAGACTGAAGAACTGATGAGAAAAATATGAGGCTCGACTCTATATTCT 2820
 Q Q A R R D P L T K Q I S D N Q I L L Q
 2821 TCAGCAAGCCCAGCGGGATCCACTCACCAACAAATTTCTGATAACCAAATACTGCTTCA 2880
 E L G P G D G I V M A F D N V L Q K L L
 2881 AGAACTGGGTCTCGAGATGGTATCGTCATGGCGTTTCGATAACGCTCTGCAGAACTCCT 2940
 E E Y G S D D T R N V K E T T E Y L K T
 2941 GGAGGAATATGGGAGTGATGACACAAGGAATGTGAAAGAAACCACAGAGTACTTAAAAAC 3000
 S W I N L K Q S I A D R Q N A L E A E W
 3001 ATCATGGATCAATCTCAAACAAAGTATTGCTGACAGACAGAACGCCTTGGAGGCTGAGTG 3060
 R T V Q A S R R D L E N F L K W I Q E A
 3061 GAGGACGGTGCAGGCCTCTCGCAGAGATCTGGAAAACTTCCTGAAGTGGATCCAAGAAGC 3120
 E T T V N V L V D A S H R E N A L Q D S
 3121 AGAGACCACAGTGAATGTGCTTGTGGATGCCTCTCATCGGGAGAATGCTCTTCAGGATAG 3180
 I L A R E L K Q Q M Q D I Q A E I D A H
 3181 TATCTTGGCCAGGGAACCTCAAACAGCAGATGCAGGACATCCAGGCAGAAATTGATGCCCA 3240
 N D I F K S I D G N R Q K M V K A L G N
 3241 CAATGACATATTAAAAAGCATTGACGGAACAGGCAGAAGATGGTAAAAGCTTTGGGAAA 3300
 S E E A T M L Q H R L D D M N Q R W N D
 3301 TTCTGAAGAGGCTACTATGCTTCAACATCGACTGGATGATATGAACCAAGATGGAATGA 3360
 L K A K S A S I R A H L E A S A E K W N
 3361 CTTAAAAGCAAAATCTGCTAGCATCAGGGCCCATTTGGAGGCCAGCGCTGAGAAGTGGAA 3420
 R L L M S L E E L I K W L N M K D E E L
 3421 CAGGTTGCTGATGTCCTTAGAAGAAGTATCAAAATGGCTGAATATGAAAGATGAAGAGCT 3480
 K K Q M P I G G D V P A L Q L Q Y D H C
 3481 TAAGAAACAAATGCCTATTGGAGGAGATGTTCCAGCCTTACAGCTCCAGTATGACCATTG 3540
 K A L R R E L K E K E Y S V L N A V D Q
 3541 TAAGGCCCTGAGACGGGAGTTAAAGGAGAAAGAATATTCTGTCTGAATGCTGTGACCA 3600
 A R V F L A D Q P I E A P E E P R R N L
 3601 GGCCCGAGTTTCTTGGCTGATCAGCCAATTGAGGCCCTGAAGAGCCAAGAAGAACT 3660
 Q S K T E L T P E E R A Q K I A K A M R
 3661 ACAATCAAAAACAGAATTAACCTCTGAGGAGAGAGCCCAAGATTGCCAAAGCCATGCG 3720

Figure 3 Continued

K Q S S E V K E K W E S L N A V T S N W
 3721 CAAACAGTCTTCTGAAGTCAAAGAAAAATGGGAAAGTCTAAATGCTGTAAGTCTAGCAATTG 3780
 Q K Q V D K A L E K L R D L Q G A M D D
 3781 GCAAAAGCAAGTGGACAAGGCATTGGAGAACTCAGAGACCTGCAGGGAGCTATGGATGA 3840
 L D A D M K E A E S V R N G W K P V G D
 3841 CCTGGACGCTGACATGAAGGAGGCAGAGTCCGTGCGGAATGGCTGGAAGCCCCGTGGGAGA 3900
 L L I D S L Q D H I E K I M A F R E E I
 3901 CTTACTCATTCGCTGCGTGCAGGATCACATTGAAAAATCATGGCATTCTAGAGAAGAAAT 3960
 A P I N F K V K T V N D L S S Q L S P L
 3961 TGCACCAATCAACTTTAAAGTTAAACGGTGAATGATTTATCCAGTCAGCTGTCTCCACT 4020
 D L H P S L K M S R Q L D D L N M R W K
 4021 TGACCTGCATCCCTCTCTAAAGATGTCTCGCCAGCTAGATGACCTTAATATGCGATGGAA 4080
 L L Q V S V D D R L K Q L Q E A H R D F
 4081 ACTTTTACAGGTTTCTGTGGATGATCGCCTTAAACAGCTTCAGGAAGCCCACAGAGATTT 4140
 G P S S Q H F L S T S V Q L P W Q R S I
 4141 TGGACCATCCTCTCAGCATTTTCTCTCTACGTTCAGTCCAGCTGCCGTGGCAAAGATCCAT 4200
 S H N K V P Y Y I N H Q T Q T T C W D H
 4201 TTCACATAATAAAGTGCCCTATTACATCAACCATCAAACACAGACCACCTGTTGGGACCA 4260
 P K M T E L F Q S L A D L N N V R F S A
 4261 TCCTAAATGACCGAATCTTTCAATCCCTTGCTGACCTGAATAATGTACGTTTTTCTGC 4320
 Y R T A I K I R R L Q K A L C L D L L E
 4321 CTACCGTACAGCAATCAAAATCCGAAGACTACAAAAGCACTATGTTTGGATCTCTTAGA 4380
 L S T T N E I F K Q H K L N Q N D Q L L
 4381 GTTGAGTACAACAAATGAAATTTTCAAACAGCACAAAGTTGAACCAAAATGACCAGCTCCT 4440
 S V P D V I N C L T T T Y D G L E Q M H
 4441 CAGTGTTCAGATGTCATCAACTGTCTGACAACAATATGATGGACTTGAGCAAATGCA 4500
 K D L V N V P L C V D M C L N W L L N V
 4501 TAAGGACCTGGTCAACGTTCCACTCTGTGTTGATATGTGTCTCAATTGGTTGCTCAATGT 4560
 Y D T G R T G K I R V Q S L K I G L M S
 4561 CTATGACACGGGTGCAACTGGAAAAATAGAGTGCAGAGTCTGAAGATTGGATTAAATGTC 4620
 L S K G L L E E K Y R Y L F K E V A G P
 4621 TCTCTCCAAAGGTCTCTTGGAAGAAAAATACAGATATCTCTTTAAGGAAGTTGCGGGGCC 4680
 T E M C D Q R Q L G L L L H D A I Q I P
 4681 GACAGAAATGTGTGACCAGAGGCAGCTGGGCCTGTTACTTCATGATGCCATCCAGATCCC 4740
 R Q L G E V A A F G G S N I E P S V R S
 4741 CCGGACGTAGGTGAAGTAGCAGCTTTTGGAGGCAGTAATATTGAGCCTAGTGTTCGCAG 4800
 C F Q Q N N N K P E I S V K E F I D W M
 4801 CTGCTTCCAACAGATAACAATAAACCAGAAATAAGTGTGAAAGAGTTTATAGATTGGAT 4860
 H L E P Q S M V W L P V L H R V A A A E
 4861 GCATTGGGAACCACAGTCCATGGTTTGGCTCCCAGTTTACATCGAGTGGCAGCAGCGGA 4920
 T A K H Q A K C N I C K E C P I V G F R
 4921 GACTGCAAAACATCAGGCCAAATGCAACATCTGTAAAGAAATGTCCAATTGTCGGGTTTCAG 4980

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Figure 3 Continued

Y R S L K H F N Y D V C Q S C F F S G R
4981 GTATAGAAGCCTTAAGCATTTTAACTATGATGCTGCCAGAGTTGTTCTTTTCGGGTGCG 5040

T A K G H K L H Y P M V E Y C I P T T S
5041 AACAGCAAAAGGTACAAAATTACATTACCCAATGGTGGAAATATTGTATACCTACAACATC 5100

G E D V R D F T K V L K N K F R S K K Y
5101 TGGGGAAGATGTACGAGACTTCACAAAGGTACTTAAGAACAAGTTCAGGTCGAAGAAGTA 5160

F A K H P R L G Y L P V Q T V L E G D N
5161 CTTTGCCAAACACCCTCGACTTGGTTACCTGCCTGTCCAGACAGTTCTTGAAGGTGACAA 5220

L E T P I T L I S M W P E H Y D P S Q S
5221 CTTAGAGACTCCTATCACACTCATCAGTATGTGGCCAGAGCACTATGACCCCTCACAATC 5280

P Q L F H D D T H S R I E Q Y A T R L A
5281 TCCTCAACTGTTTCATGATGACACCCATTCAAGAATAGAACAAATATGCCACACGACTGGC 5340

Q M E R T N G S F L T D S S S T T G S V
5341 CCAGATGGAAAGGACTAATGGGTCTTTTCTCACTGATAGCAGCTCCACCACAGGAAGTGT 5400

E D E H A L I Q Q Y C Q T L G G E S P V
5401 GGAAGACGAGCAGCCCTCATCCAGCAGTATTGCCAAACACTCGGAGGAGAGTCCCCAGT 5460

S Q P Q S P A Q I L K S V E R E E R G E
5461 GAGCCAGCCGAGAGCCAGCTCAGATCCTGAAGTCAGTAGAGAGGGAAGAACGTGGAGA 5520

L E R I I A D L E E E Q R N L Q V E Y E
5521 ACTGGAGAGGATCATTGCTGACCTGGAGGAAGAACAAGAAATCTACAGGTGGAGTATGA 5580

Q L K D Q H L R R G L P V G S P P E S I
5581 GCAGCTGAAGGACCAGCACCTCCGAAGGGGGCTCCCTGTGCGTTACCGCCAGAGTCGAT 5640

I S P H H T S E D S E L I A E A K L L R
5641 TATATCTCCCCATCACACGTCTGAGGATTGAGAACTTATAGCAGAAGCAAACTCCTCAG 5700

Q H K G R L E A R M Q I L E D H N K Q L
5701 GCAGCACAAAGGTGCGCTGGAGGCTAGGATGCAGATTTTAGAAGATCACAATAAACAGCT 5760

E S Q L H R L R Q L L E Q P E S D S R I
5761 GGAGTCTCAGCTCCACCGCTCCGACAGCTGCTGGAGCAGCCTGAATCTGATTCCCGAAT 5820

N G V S P W A S P Q H S A L S Y S L D P
5821 CAATGGTGTTTCCCCATGGGCTTCTCCTCAGCATTCTGCACTGAGCTACTCGCTTGATCC 5880

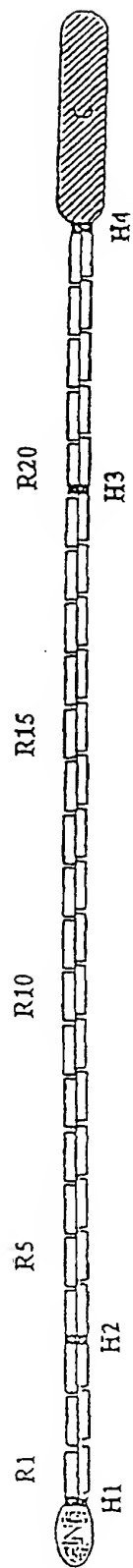
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5881 AGATGCCTCCGGCCACAGTTCCACCAGGCAGCGGAGAGGACCTGCTGGCCCCACCGCA 5940

D T S T D L T E V M E Q I H S T F P S C
5941 CGACACCAGCAGGATCTCAGGAGGTATGGAGCAGATTACAGCACGTTTCCATCTTG 6000

C P N V P S R P Q A M *
6001 CTGCCCCAATGTTCCAGCAGGCCACAGGCAATGTAATCACTAGT 6045

Figure 4

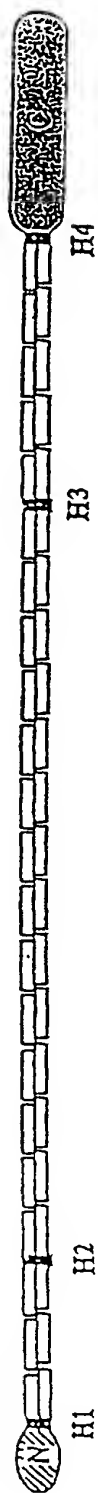
Dystrophin



Dystrophin minigene



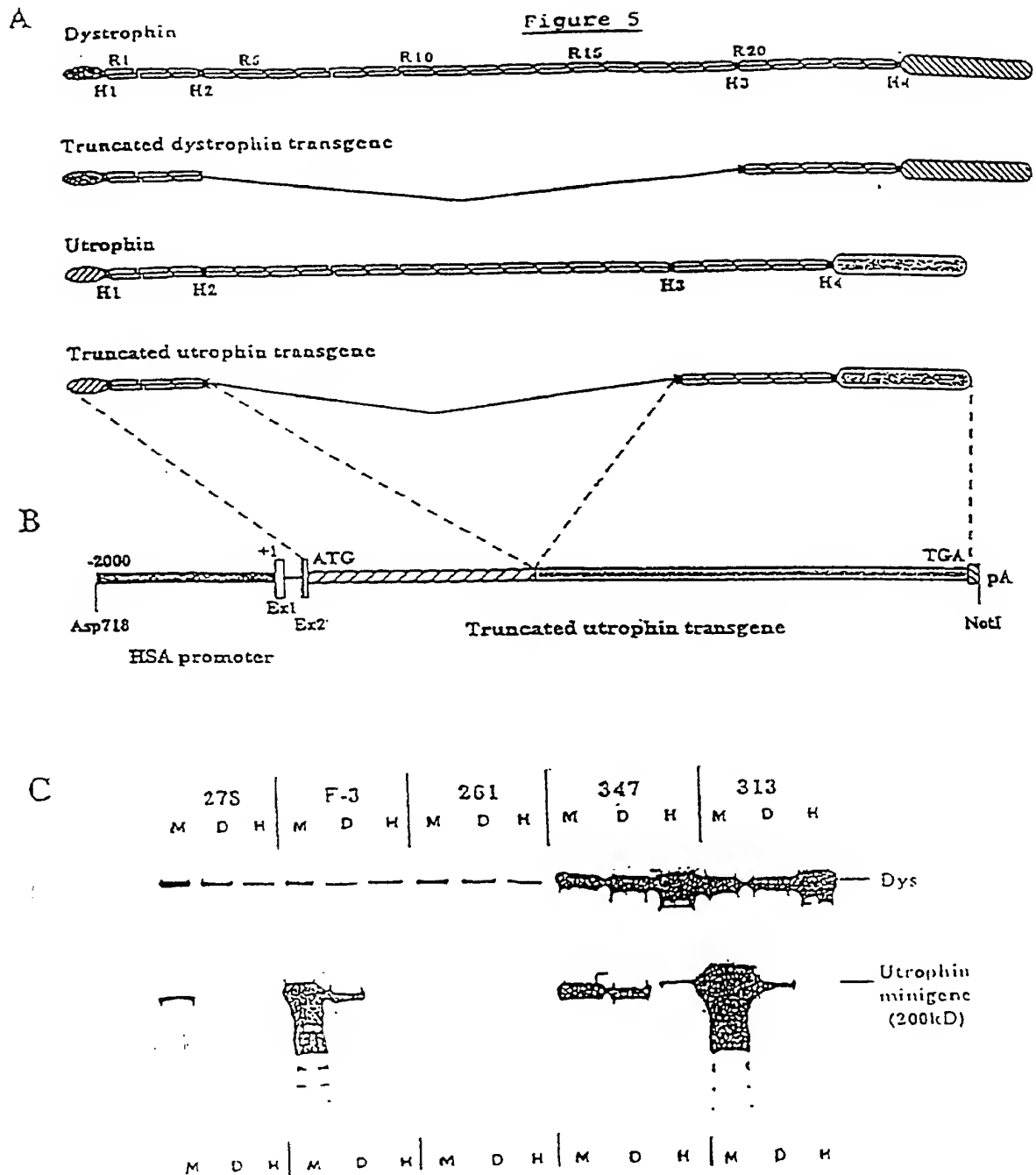
Utrophin



Utrophin PCR minigene



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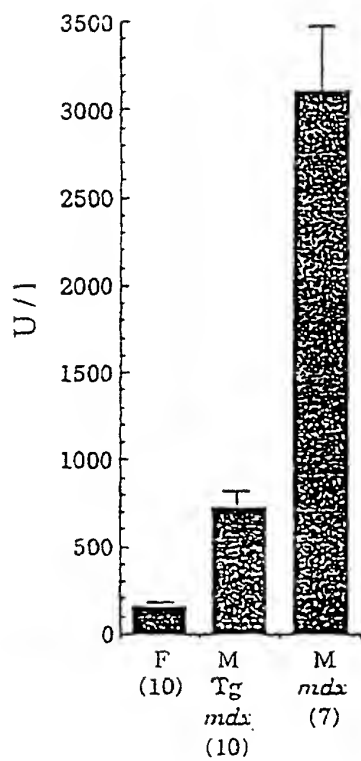


SUBSTITUTE SHEET (RULE 26)

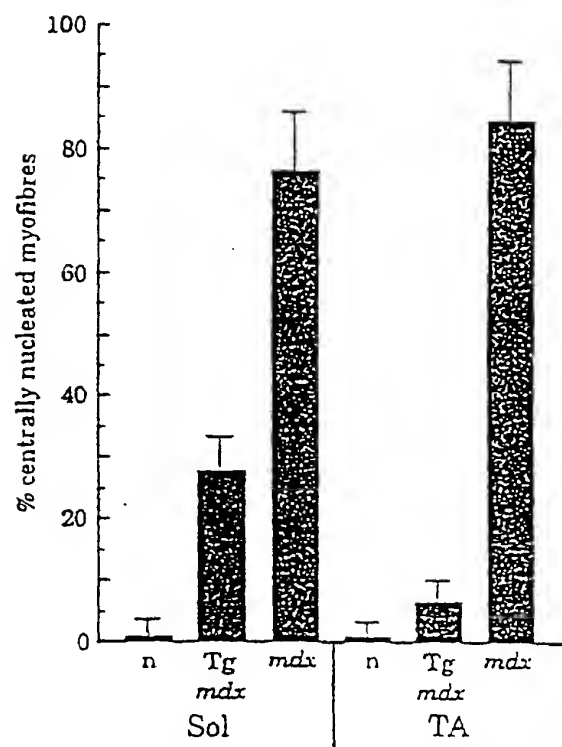
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Figure 6

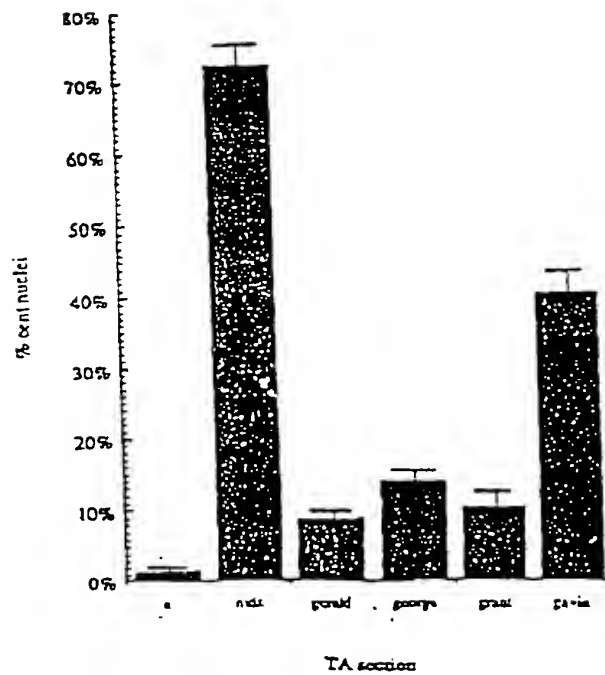
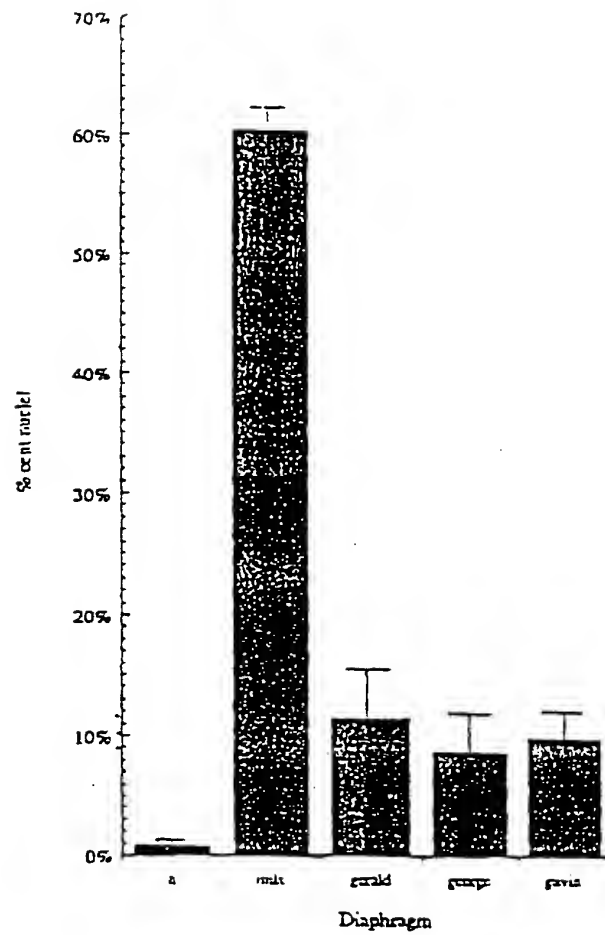
A



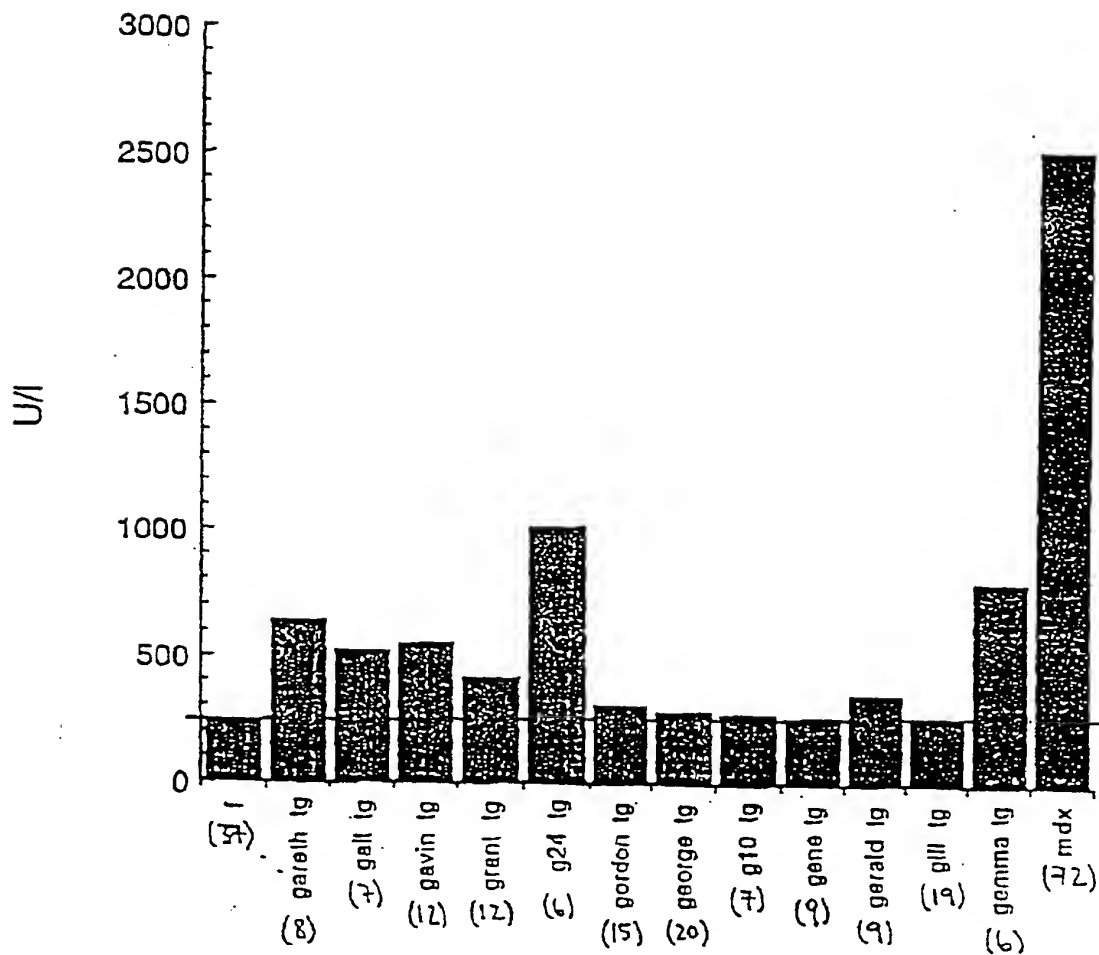
B



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Figure 7

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Figure 8

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Figure 9

M A K Y G E H E A S P D N G Q N E
 1 ACTAGTCAAGATGGCCAAGTATGGAGAACATGAAGCCAGTCCTGACAATGGGCAGAACGA 60
 F S D I I E S R S D E H N D V Q K K T F
 61 ATTCAGTGACATCATTGAGTCCAGATCTGATGAACACAATGATGTACAGAAGAAAACCTT 120
 T K W I N A R F S K S G K P P I S D M F
 121 TACCAAATGGATAAACGCTCGATTTTCCAAGAGTGGGAAACCACCCATCAGTGATATGTT 180
 S D L K D G R K L L D L L E G L T G T S
 181 CTCAGACCTCAAAGATGGGAGAAAGCTCTTGGATCTTCTCGAAGGCCCTCACAGGAACATC 240
 L P K E R G S T R V H A L N N V N R V L
 241 ATTGCCAAAGGAACGTGGTTCCACAAGGGTGCATGCCTTAAACAATGTCAACCGAGTGCT 300
 Q V L H Q N N V D L V N I G G T D I V D
 301 ACAGGTTTTACATCAGAACAATGTGGACTTGGTGAATATTGGAGGCACGGACATTGTGGA 360
 G N P K L T L G L L W S I I L H W Q V K
 361 TGGAAATCCCAAGCTGACTTTAGGGTTACTCTGGAGCATCATCTCTGCACTGGCAGGTGAA 420
 D V M K D I M S D L Q Q T N S E K I L L
 421 GGATGTCATGAAAGATATCATGTCTAGACCTGCAGCAGACAAACAGCGAGAAGATCCTGCT 480
 S W V R Q T T R P Y S Q V N V L N F T T
 481 GAGCTGGGTGCGGCAGACCACCAGGCCCTACAGTCAAGTCAACGTCTCACTTCAACCAC 540
 S W T D G L A F N A V L H R H K P D L F
 541 CAGCTGGACCGATGGACTCGCGTTCAACGCCGTGCTCCACCGGCACAAACCAGATCTCTT 600
 S W D R V V K M S P I E R L E H A F S K
 601 CAGCTGGGACAGAGTGGTCAAATGTCCCCAATTGAGAGACTTGAACATGCTTTTAGCAA 660
 A H T Y L G I E K L L D P E D V A V H L
 661 GGCCACACTTATTTGGGAATTGAAAAGCTTCTAGATCCTGAAGATGTTGCTGTGCATCT 720
 P X X X X X X X X X X X X V E V L P Q Q
 721 CCCNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNCCGTTGAGGTGCTTCCTCAGCA 780
 V T I D A I R E V E T L P R K Y K K E C
 781 AGTCACGATAGATGCCATCCGAGAGGTGGAGACTCTCCCAAGGAAGTATAAGAAAGAATG 840
 E E E E I H I Q S A V L A E E G Q S P R
 841 TGAAGAGGAAGAAATTCATATCCAGAGTGCAGTGCTGGCAGAGGAAGGCCAGAGTCCCCG 900
 A E T P S T V T E V D M D L D S Y Q I A
 901 AGCTGAGACCCCTAGCACCGTCACTGAAGTGGACATGGATTTGGACAGCTACCAGATAGC 960
 L E E V L T W L L S A E D T F Q E Q D D
 961 GCTAGAGGAAGTGTGCTGACGTGGCTGCTGTCCGCGGAGGACACGTTCCAGGAGCAAGATGA 1020
 I S D D V E E V K E Q F A T H E T F M M
 1021 CATTTCTGATGATGTGGAAGAAGTCAAAGAGCAGTTTGCTACCCATGAAACTTTTATGAT 1080
 E L T A H Q S S V G S V L Q A G N Q L M
 1081 GGAGCTGACAGCACACCAGAGCAGCGTGGGAGCGTCTGTCAGGCTGGCAACCAGCTGAT 1140
 T Q G T L S E E E E F E I Q E Q M T L L
 1141 GACACAAGGGACTCTGTCAGAGGAGGAGGAGTTTGTAGATCCAGGAACAGATGACCTTGCT 1200

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Figure 9 Continued

N A R W E A L R V E S M E R Q S R L H D
 1201 GAATGCAAGGTGGGAGGCGCTCCGGGTGGAGAGCATGGAGAGGCAGTCCCGGCTGCACGA 1260
 A L M E L Q K K Q L Q Q L S S W L A L T
 1261 CGCTCTGATGGAGCTGCAGAAGAAACAGCTGCAGCAGCTCTCAAGCTGGCTGGCCCTCAC 1320
 E E R I Q K M E S P P L G D D L P S L Q
 1321 AGAAGAGCGCATTTCAGAAGATGGAGAGCCCTCCGCTGGGTGATGACCTGCCCTCCCTGCA 1380
 K L L Q E H K S L Q N D L E A E Q V K V
 1381 GAAGCTGCTTCAAGAACATAAAAGTTTGCAAAATGACCTTGAAGCTGAACAGGTGAAGGT 1440
 N S L T H M V V I V D E N S G E S A T A
 1441 AAATTCCTTAACTCACATGGTGGTGATTGTGGATGAAAACAGTGGGAGAGTGCCACAGC 1500
 L L E D Q L Q K L G E R W T A V C R W T
 1501 TCTTCTGGAAGATCAGTTACAGAACTGGGTGAGCGCTGGACAGCTGTATGCCGCTGGAC 1560
 E E R W N R L Q E I S I L W Q E L L E E
 1561 TGAAGAAGCTTGGAAACAGGTTGCAAGAAATCAGTATTCTGTGGCAGGAATTATTGGAAGA 1620
 Q C L L E A W L T E K E E A L N K V Q T
 1621 GCAGTGTCTGTGGAGGCTTGGCTCACCAGAAAGGAAGAGGCTTTGAATAAAGTTCAAAC 1680
 S N F K D Q K E L S V S V R R L A I L K
 1681 CAGCAACTTTAAAGACCAGAAGGAAGTAAGTGTGAGTGTCCGGCGTCTGGCTATATTGAA 1740
 E D M E M K R Q T L D Q L S E I G Q D V
 1741 GGAAGACATGGAATGAAGAGGCAGACTCTGGATCAACTGAGTGAGATTGGCCAGGATGT 1800
 G Q L L S N P K A S K K M N S D S E E L
 1801 GGGCCAATTACTCAGTAATCCCAAGGCATCTAAGAAGATGAACAGTGACTCTGAGGAGCT 1860
 T Q R W D S L V Q R L E D S S N Q V T Q
 1861 AACACAGAGATGGGATTCTCTGGTTTCAGAGACTCGAAGACTCTTCTAACCAGGTGACTCA 1920
 A V A K L G M S Q I P Q K D L L E T V H
 1921 GGCGGTAGCGAAGCTCGGCATGTCCCAGATTCCACAGAAGGACCTATTGGAGACCGTTCA 1980
 V R E K G M V K K P K Q E L P P P L G P
 1981 TGTGAGAGAAAAAGGGATGGTGAAGAAGCCCAAGCAGGAAGTGCCTCCTCCGTGGGGCC 2040
 K K R Q I H V D I E A K K K F D A I S A
 2041 AAAGAAGAGACAGATCCATGTGGATATTGAAGCTAAGAAAAAGTTTGATGCTATAAGTGC 2100
 E L L N W I L K W K T A I Q T T E I K E
 2101 AGAGCTGTTGAACTGGATTTTGAAATGGAAGAACTGCCATTACACCACAGAGATAAAAGA 2160
 Y M K M Q D T S E M K K K L K A L E K E
 2161 GTATATGAAGATGCAAGACACTCCGAAATGAAAAAGAAGTTGAAGGCATTAGAAAAAGA 2220
 Q R E R I P R A D E L N Q T G Q I L V E
 2221 ACAGAGAGAAAGAATCCCCAGAGCAGATGAATTAACCAAACTGGACAAATCCTTGTGGA 2280
 Q M G K E G L P T E E I K N V L E K V S
 2281 GCAAAATGGGAAAAAGAGGCCTTCTACTGAAGAAATAAAAAATGTTCTGGAGAAGGTTTC 2340
 S E W K N V S Q H L E D L E R K I Q L Q
 2341 ATCAGAATGGAAGAATGTATCTCAACATTTGGAAGATCTAGAAAGAAAGATTGAGCTACA 2400
 E D I N A Y F K Q L D E L E K V I K T K
 2401 GGAAGATATAAATGCTTATTTCAGCAGCTTGATGAGCTTGAAGGTCATCAAGACAAA 2460

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Figure 9 Continued

E E W V K H T S I S E S S R Q S L P S L
 2461 GGAGGAGTGGGTAAAAACACACTTCCATTTCTGAATCTTCCCGGCAGTCCTTGCCAAGCTT 2520
 K D S C Q R E L T N L L G L H P K I E M
 2521 GAAGGATTCTCTGCAGCGGGAATTGACAAATCTTCTTGGCCTTCACCCCAAATTTGAAT 2580
 A R A S C S A L M S Q P S A P D F V Q R
 2581 GGCTCGTGCAAGCTGCTCGGCCCTGATGTCTCAGCCTTCTGCCCCAGATTTGTCCAGCG 2640
 G F D S F L G R Y Q A V Q E A V E D R Q
 2641 GGGCTTCGATAGCTTTCTGGGCCGCTACCAAGCTGTACAAGAGGCTGTAGAGGATCGTCA 2700
 Q H L E N E L K G Q P G H A Y L E T L K
 2701 ACAACATCTAGAGAATGAACTGAAGGGCCAACTGGACATGCATATCTGGAAACATTGAA 2760
 T L K D V L N D S E N K A Q V S L N V L
 2761 AACACTGAAAGATGTGCTAAATGATTGAGAAAATAAGGCCAGGTGTCTCTGAATGTCCT 2820
 N D L A K V E K A L Q E K K T L D E I L
 2821 TAATGATCTTGCCAAGGTGGAGAAGGCCCTGCAAGAAAAAAGACCCCTTGATGAAATCCT 2880
 E N Q K P A L H K L A E E T K A L E K N
 2881 TGAGAATCAGAAACCTGCATTACATAAATTTGCAGAAGAAACAAAGGCTCTGGAGAAAA 2940
 V H P D V E K L Y K Q E F D D V Q G K W
 2941 TGTTCATCCTGATGTAGAAAAATTATATAAGCAAGAATTTGATGATGTGCAAGGAAAGTG 3000
 N K L K V L V S K D L H L L E E I A L T
 3001 GAACAAGCTAAAGGTCTTGGTTTCCAAAGATCTACATTTGCTTGAGGAAATTGCTCTCAC 3060
 L R A F E A D S T V I E K W M D G V K D
 3061 ACTCAGAGCTTTTGAGGCCGATTCAACAGTCATTGAGAAGTGATGGATGGCGTGAAAGA 3120
 F L M K Q Q A A Q G D D A G L Q R Q L D
 3121 CTTCTTAATGAAACAGCAGGCTGCCCAAGGAGACGACGCGGTCTACAGAGGCAGTTAGA 3180
 Q C S A F V N E I E T I E S S L K N M K
 3181 CCAGTGCTCTGCATTTGTTAATGAAATAGAAACAATTGAATCATCTCTGAAAAACATGAA 3240
 E I E T N L R S G P V A G I K T W V Q T
 3241 GGAAATAGAGACTAATCTTCGAAGTGGTCCAGTTGCTGGAATAAAAACTTGGGTGCAGAC 3300
 R L G D Y Q T Q L E K L S K E I A T Q K
 3301 AAGACTAGGTGACTACCAAACCTCAACTGGAGAACTTAGCAAGGAGATCGCTACTCAAAA 3360
 S R L S E S Q E K A A N L K K D L A E M
 3361 AAGTAGGTTGTCTGAAAGTCAAGAAAAAGCTGCGAACCTGAAGAAAGACTTGGCAGAGAT 3420
 Q E W M T Q A E E E Y L E R D F E Y K S
 3421 GCAGGAATGGATGACCCAGGCCGAGGAAGAAATATTTGGAGCGGGATTTTGAGTACAAGTC 3480
 P E E L E S A V E E M K R A K E D V L Q
 3481 ACCAGAAGAGCTTGAGAGTGTGTGGAAGAGATGAAGAGGGCAAAAGAGGATGTGTTGCA 3540
 K E V R V K I L K D N I K L L A A K V P
 3541 GAAGGAGGTGAGAGTGAAGATTCTCAAGGACAACATCAAGTTATTAGCTGCCAAGGTGCC 3600
 S G G Q E L T S E L N V V L E N Y Q L L
 3601 CTCTGGTGGCCAGGAGTTGACCTCTGAGCTGAATGTTGTGCTGGAGAATTACCAACTTCT 3660
 C N R I R G K C H T L E E V W S C W I E
 3661 TTGTAATAGAATTGAGGAAAGTGCCACACGCTAgagGAGGTCTGGTCTGTTGGATTGA 3720

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Figure 9 Continued

L L H Y L D L E T T W L N T L E E R M K
 3721 ACTGCTTCACTATTTGGATCTTGAAACTACCTGGTTAAACACTTTGGAAGAGCGGATGAA 3780
 S T E V L P E K T D A V N E A L E S L E
 3781 GAGCACAGAGGTCCTGCCTGAGAAGACGGATGCTGTCAACGAAGCCCTGGAGTCTCTGGA 3840
 S V L R H P A D N R T Q I R E L G Q T L
 3841 ATCTGTTCTGCGCCACCCGGCAGATAATCGCACCCAGATTGAGAGCTTGGCCAGACTCT 3900
 I D G G I L D D I I S E K L E A F N S R
 3901 GATTGATGGGGGATCCTGGATGATATAATCAGTGAGAACTGGAGGCTTTCAACAGCCG 3960
 Y E D L S H L A E S K Q I S L E K Q L Q
 3961 ATATGAAGATCTAAGTCACCTGGCAGAGAGCAAGCAGATTTCTTTGGAAGCAACTCCA 4020
 V L R E T D Q M L Q V L Q E S L G E L D
 4021 GGTGCTGCGGGAACTGACCAGATGCTTCAAGTCTTGCAAGAGAGCTTGGGGGAGCTGGA 4080
 K Q L T T Y L T D R I D A F Q V P Q E A
 4081 CAAACAGCTCACCACATACCTGACTGACAGGATAGATGCTTTCCAAGTTCCACAGGAAGC 4140
 Q K I Q A E I S A H E L T L E E L R R N
 4141 TCAGAAAATCCAAGCAGAGATCTCAGCCCATGAGCTAACCCTAGAGGAGTTGAGAAGAAA 4200
 M R S Q P L T S P E S R T A R G G S Q M
 4201 TATGCGTTCTCAGCCCTGACCTCCCCAGAGAGTAGGACTGCCAGAGGAGGAAGTCAGAT 4260
 D V L Q R K L R E V S T K F Q L F Q K P
 4261 GGATGTGCTACAGAGGAACTCCGAGAGGTGTCACAAAGTTCCAGCTTTTCCAGAAGCC 4320
 A N F E Q R M L D C K R V L D G V K A E
 4321 AGCTAACTTCGAGCAGCGCATGCTGGACTGCAAGCGTGTGCTGGATGGCGTGAAAGCAGA 4380
 L H V L D V K D V D P D V I Q T H L D K
 4381 ACTTCACGTTCTGGATGTGAAGGACGTAGACCCTGACGTCATACAGACGCACCTGGACAA 4440
 C M K L Y K T L S E V K L E V E T V I K
 4441 GTGTATGAACTGTATAAACTTTGAGTGAAGTCAAACCTGAAGTGGAAGTGTGATTAA 4500
 T G R H I V Q K Q Q T D N P K G M D E Q
 4501 AACAGGAAGACATATTGTCCAGAAAACAGCAAAACGGACAACCCAAAAGGGATGGATGAGCA 4560
 L T S L K V L Y N D L G A Q V T E G K Q
 4561 GCTGACTTCCCTGAAGGTTCTTTACAATGACCTGGGCGCACAGGTGACAGAAGGAAAACA 4620
 D L E R A S Q L A R K M K K E A A S L S
 4621 GGATCTGGAAGAGCATCACAGTTGGCCCCGAAAATGAAGAAAGAGGCTGCTTCTCTCTC 4680
 E W L S A T E T E L V Q K S T S E G L L
 4681 TGAATGGCTTTCTGCTACTGAACTGAATTGGTACAGAAGTCCACTTCAGAAGGTCTGCT 4740
 G D L D T E I S W A K N V L K D L E K R
 4741 TGGTGACTTGGATACAGAAATTTCTGGGGCTAAAAATGTTCTGAAGGATCTGGAAAAGAG 4800
 K A D L N T I T E S S A A L Q N L I E G
 4801 AAAAGCTGATTTAAATACCATCACAGAGAGTAGTGCTGCCCTGCAAACTTGATTGAGGG 4860
 S E P I L E E R L C V L N A G W S R V R
 4861 CAGTGAGCCTATTTAGAAGAGAGGCTCTGCGTCTTAACGCTGGGTGGAGCCGAGTTCC 4920
 T W T E D W C N T L M N H Q N Q L E I F
 4921 TACCTGGACTGAAGATTGGTGCAATACCTTGATGAACCATCAGAACCAGCTAGAAATATT 4980

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Figure 9 Continued

D G N V A H I S T W L Y Q A E A L L D E
 4981 TGATGGGAACGTGGCTCACATAAGTACCTGGCTTTATCAAGCTGAAGCTCTATTGGATGA 5040
 I E K K P T S K Q E E I V K R L V S E L
 5041 AATTGAAAAGAAACCAACAAGTAAACAGGAAGAAATTGTGAAGCGTTTAGTATCTGAGCT 5100
 D D A N L Q V E N V R D Q A L I L M N A
 5101 GGATGATGCCAACCTCCAGGTTGAAAATGTCCGCGATCAAGCCCTTATTTTGATGAATGC 5160
 R G S S S R E L V E P K L A E L N R N F
 5161 CCGTGGGAAGCTCAAGCAGGGAGCTTGTTAGAACCAAGTTAGCTGAGCTGAATAGGAAGTT 5220
 E K V S Q H I K S A K L L I A Q E P L Y
 5221 TGAAAAGGTGTCTCAACATATCAAAAGTGCCAAATTGCTAATTGCTCAGGAACCATTTATA 5280
 Q C L V T T E T F E T G V P F S D L E K
 5281 CCAATGTTTGGTCACCACTGAAACATTTGAAACTGGTGTGCCTTTCTCTGACTTGGAAAA 5340
 L E N D I E N M L K F V E K H L E S S D
 5341 ATTAGAAAATGACATAGAAAATATGTTAAAATTTGTGGAAAAACACTTGGAAATCCAGTGA 5400
 E D E K M D E E S A Q I E E V L Q R G E
 5401 TGAAGATGAAAAGATGGATGAGGAGAGTGCCAGATTGAGGAAGTTCTACAAAGAGGAGA 5460
 E M L H Q P M E D N K K E K I R L Q L L
 5461 AGAAATGTTACATCAACCTATGGAAGATAATAAAAAAGAAAGATCCGTTTGCAATTATT 5520
 L L H T R Y N K I K A I P I Q Q R K M G
 5521 ACTTTTGCATACTAGATACAACAAAATTAAGGCAATCCCTATTCAACAGAGGAAAATGGG 5580
 Q L A S G I R S S L L P T D Y L V E I N
 5581 TCAACTTGCTTCTGGAATTAGATCATCACTTCTTCTACAGATTATCTGGTTGAAATTAA 5640
 K I L L C M D D V E L S L N V P E L N T
 5641 CAAAATTTTACTTTGCATGGATGATGTTGAATTATCGCTTAATGTTCCAGAGCTCAACAC 5700
 A I Y E D F S F Q E D S L K N I K D Q L
 5701 TGCTATTTACGAAGACTTCTCTTTTCAGGAAGACTCTCTGAAGAATATCAAAGACCAACT 5760
 D K L G E Q I A V I H E K Q P D V I L E
 5761 GGACAACTTGGAGAGCAGATTGCAGTCATTCATGAAAAACAGCCAGATGTCATCCTTGA 5820
 A S G P E A I Q I R D T L T Q L N A K W
 5821 AGCCTCTGGACCTGAAGCCATTGAGATCAGAGATACACTTACTCAGCTGAATGCAAAATG 5880
 D R I N R M Y S D R K G C F D R A M E E
 5881 GGACAGAATTAATAGAATGTACAGTGATCGGAAAGGTTGTTTGGACAGGGCAATGGAAGA 5940
 W R Q F H C D L N D L T Q W I T E A E E
 5941 ATGGAGACAGTTCATTGTGACCTTAATGACCTCACACAGTGGATAACAGAGGCTGAAGA 6000
 L L V D T C A P G G S L D L E K A R I H
 6001 ATTACTGGTTGATACCTGTGCTCCAGGTGGCAGCCTGGACTTAGAGAAAGCCAGGATACA 6060
 Q Q E L E V G I S S H Q P S F A A L N R
 6061 TCAGCAGGAACCTGAGGTGGGCATCAGCAGCCACCAGCCAGTTTTCAGCACTAAACCG 6120
 T G D G I V Q K L S Q A D G S F L K E K
 6121 AACTGGGGATGGGATTGTGCAGAACTCTCCAGGCAGATGGAAGCTTCTTGAAAGAAAA 6180
 L A G L N Q R W D A I V A E V K D R Q P
 6181 ACTGCGAGGTTTAAACCAACGCTGGGATGCAATTGTTGCAGAAGTGAAGGATAGGCAGCC 6240

Figure 9 Continued

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      R L K G E S K Q V M K Y R H Q L D E I I
6241 AAGGCTAAAAGGAGAAAGTAAGCAGGTGATGAAGTACAGGCATCAGCTAGATGAGATTAT 6300

      C W L T K A E H A M Q K R S T T E L G E
6301 CTGTTGGTTAAACAAAGGCTGAGCATGCTATGCAAAAGAGATCAACCACCGAATTGGGAGA 6360

      N L Q E L R D L T Q E M E V H A E K L K
6361 AAACCTGCAAGAATTAAGAGACTTAACTCAAGAAATGGAAGTACATGCTGAAAAACTCAA 6420

      W L N R T E L E M L S D K S L S L P E R
6421 ATGGCTGAATAGAACTGAATTGGAGATGCTTTCAGATAAAAGTCTGAGTTTACCTGAAAG 6480

      D K I S E S L R T V N M T W N K I C R E
6481 GGATAAAATTTTCAGAAAGCTTAAGGACTGTAAATATGACATGGAATAAGATTTCAGAGA 6540

      V P T T L K E C I Q E P S S V S Q T R I
6541 GGTGCCTACCACCTGAAGGAATGCATCCAGGAGCCAGTTCTGTTTCACAGACAAGGAT 6600

      A A H P N V Q K V V L V S S A S D I P V
6601 TGCTGCTCATCCTAATGTCCAAAAGGTGGTGTAGTATCATCTGCGTCAGATATTCCTGT 6660

      Q S H R T S E I S I P A D L D K T I T E
6661 TCAGTCTCATCGTACTTCGGAAATTTCAATTCCTGCTGATCTTGATAAACTATAACAGA 6720

      L A D W L V L I D Q M L K S N I V T V G
6721 ACTAGCCGACTGGCTGGTATTAAATCGACCAGATGCTGAAGTCCAACATTGTCACTGTTGG 6780

      D V E E I N K T V S R M K I T K A D L E
6781 GGATGTAGAAGAGATCAATAAGACCGTTTCCCGAATGAAAATTACAAAGGCTGACTTAGA 6840

      Q R H P Q L D Y V F T L A Q N L K N K A
6841 ACACGCCCATCTCAGCTGGATTATGTTTTTACATTGGCACAGAATTTGAAAAATAAAGC 6900

      S S S D M R T A I T E K L E R V K N Q W
6901 TTCCAGTTCAGATATGAGAACAGCAATTACAGAAAAATTGAAAAGGGTCAAGAACCAGTG 6960

      D G T Q H G V E L R Q Q Q L E D M I I D
6961 GGATGGCACCCAGCATGGCGTTGAGCTAAGACAGCAGCAGCTTGAGGACATGATTATTGA 7020

      S L Q W D D H R E E T E E L M R K Y E A
7021 CAGTCTTCAGTGGGATGACCATAGGGAGGAGACTGAAGAAGTATGAGAAAATATGAGGC 7080

      R L Y I L Q Q A R R D P L T K Q I S D N
7081 TCGACTCTATATTCTTCAGCAAGCCCGACGGGATCCACTCACCAACAAATTTCTGATAA 7140

      Q I L L Q E L G P G D G I V M A F D N V
7141 CCAAACTAGCTTCAAGAACTGGGTCTGGAGATGGTATCGTCATGGCGTTTCGATAACGT 7200

      L Q K L L E E Y G S D D T R N V K E T T
7201 CCTGCAGAACTCCTGGAGGAATATGGGAGTGATGACACAAGGAATGTGAAAGAAACCAC 7260

      E Y L K T S W I N L K Q S I A D R Q N A
7261 AGAGTACTTAAAAACATCATGGATCAATCTCAAACAAAGTATTGCTGACAGACAGAACGC 7320

      L E A E W R T V Q A S R R D L E N F L K
7321 CTTGGAGGCTGAGTGGAGGACGGTGCAGGCCTCTCGCAGAGATCTGGAAAACCTCCTGAA 7380

      W I Q E A E T T V N V L V D A S H R E N
7381 GTGGATCCAAGAAGCAGAGACCACAGTGAATGTGCTTGTGGATGCCTCTCATCGGGAGAA 7440

      A L Q D S I L A R E L K Q Q M Q D I Q A
7441 TGCTCTTCAGGATAGTATCTTGGCCAGGGAAGTCAAACAGCAGATGCAGGACATCCAGGC 7500

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Figure 9 Continued

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E I D A H N D I F K S I D G N R Q K M V
 7501 AGAAATTGATGCCCACAATGACATATTTAAAAGCATTGACGGAAACAGGCAGAAGATGGT 7560
 K A L G N S E E A T M L Q H R L D D M N
 7561 AAAAGCTTTGGGAAATTCTGAAGAGGCTACTATGCTTCAACATCGACTGGATGATATGAA 7620
 Q R W N D L K A K S A S I R A H L E A S
 7621 CCAAAGATGGAATGACTTAAAAGCAAATCTGCTAGCATCAGGGCCCATTGGAGGCCAG 7680
 A E K W N R L L M S L E E L I K W L N M
 7681 CGCTGAGAAGTGGAAACAGGTTGCTGATGTCCTTAGAAGAACTGATCAAATGGCTGAATAT 7740
 K D E E L K K Q M P I G G D V P A L Q L
 7741 GAAAGATGAAGAGCTTAAGAAACAAATGCCTATTGGAGGAGATGTTCCAGCCTTACAGCT 7800
 Q Y D H C K A L R R E L K E K E Y S V L
 7801 CCAGTATGACCATTGTAAGGCCCTGAGACGGGAGTTAAAGGAGAAAGAAATATTCTGTCCT 7860
 N A V D Q A R V F L A D Q P I E A P E E
 7861 GAATGCTGTCGACCAGGCCCGAGTTTCTTGGCTGATCAGCCAATTGAGGCCCTGAAGA 7920
 P R R N L Q S K T E L T P E E R A Q K I
 7921 GCCAAGAAGAAACCTACAATCAAAAACAGAATTAACCTCTGAGGAGAGAGCCCAAAAGAT 7980
 A K A M R K Q S S E V K E K W E S L N A
 7981 TGCCAAAGCCATGCGCAAACAGTCTTCTGAAGTCAAAGAAAAATGGGAAAGTCTAAATGC 8040
 V T S N W Q K Q V D K A L E K L R D L Q
 8041 TGTAAGTAGCAATTGGCAAAAGCAAGTGGACAAGGCATTGGAGAAACTCAGAGACCTGCA 8100
 G A M D D L D A D M K E A E S V R N G W
 8101 GGGAGCTATGGATGACCTGGACGCTGACATGAAGGAGGCAGAGTCCGTGCGGAATGGCTG 8160
 K P V G D L L I D S L Q D H I E K I M A
 8161 GAAGCCCGTGGGAGACTTACTCATTGACTCGCTGCAGGATCACATTGAAAAAATCATGGC 8220
 F R E E I A P I N F K V K T V N D L S S
 8221 ATTTAGAGAAGAAATTGCACCAATCAACTTTAAAGTTAAAACGGTGAATGATTATCCAG 8280
 Q L S P L D L H P S L K M S R Q L D D L
 8281 TCAGCTGTCTCCACTTGACCTGCATCCCTCTCTAAAGATGTCTCGCCAGCTAGATGACCT 8340
 N M R W K L L Q V S V D D R L K Q L Q E
 8341 TAATATGCGATGGAACTTTTACAGGTTTCTGTGGATGATCGCCTTAAACAGCTTCAGGA 8400
 A H R D F G P S S Q H F L S T S V Q L P
 8401 AGCCACAGAGATTTTGGACCATCCTCTCAGCATTTTCTCTCTACGTGAGTCCAGCTGCC 8460
 W Q R S I S H N K V P Y Y I N H Q T Q T
 8461 GTGGCAAAGATCCATTTACATAATAAAGTGCCTATTACATCAACCATCAAACACAGAC 8520
 T C W D H P K M T E L F Q S L A D L N N
 8521 CACCTGTGGGACCATCCTAAAATGACCGAAGCTTTTCAATCCCTTGCTGACCTGAATAA 8580
 V R F S A Y R T A I K I R R L Q K A L C
 8581 TGTACGTTTTTCTGCCTACCGTACAGCAATCAAAATCCGAAGACTACAAAAAGCACTATG 8640
 L D L L E L S T T N E I F K Q H K L N Q
 8641 TTTGGATCTCTTAGAGTTGAGTACAACAAATGAAATTTTCAAACAGCACAAGTTGAACCA 8700
 N D Q L L S V P D V I N C L T T T Y D G
 8701 AAATGACCAGCTCCTCAGTGTTCAGATGTTCATCAACTGTCTGACAACAACCTATGATGG 8760

Figure 9 Continued

21/23

L E Q M H K D L V N V P L C V D M C L N
 8761 ACTTGAGCAAAATGCATAAGGACCTGGTCAACGTTCCACTCTGTGTTGATATGTGTCTCAA 8820
 W L L N V Y D T G R T G K I R V Q S L K
 8821 TTGGTTGCTCAATGTCTATGACACGGGTGGAACCTGGAAAAATTAGAGTGCAGAGTCTGAA 8880
 I G L M S L S K G L L E E K Y R Y L F K
 8881 GATTGGATTAATGTCTCTCTCCAAAGGTCTCTTGAAGAAAAATACAGATATCTCTTTAA 8940
 E V A G P T E M C D Q R Q L G L L L H D
 8941 GGAAGTTGCGGGGCGACAGAAATGTGTGACCAGAGGCAGCTGGGCCTGTTACTTTCATGA 9000
 A I Q I P R Q L G E V A A F G G S N I E
 9001 TGCCATCCAGATCCCCGGCAGCTAGGTGAAGTAGCAGCTTTTGGAGGCAGTAATATTGA 9060
 P S V R S C F Q Q N N N K P E I S V K E
 9061 GCCTAGTGTTCGCAGCTGCTTCCAACAGAATAACAATAAACCAGAAATAAGTGTGAAGA 9120
 F I D W M H L E P Q S M V W L P V L H R
 9121 GTTTATAGATTGGATGCATTTGGAACCACAGTCCATGGTTTGGCTCCAGTTTACATCG 9180
 V A A A E T A K H Q A K C N I C K E C P
 9181 AGTGGCAGCAGCGGAGACTGCAAAACATCAGGCCAAATGCAACATCTGTAAAGAATGTCC 9240
 I V G F R Y R S L K H F N Y D V C Q S C
 9241 AATTGTCGGGTTTCAGGTATAGAAGCCTTAAGCATTTTAACATATGATGTCTGCCAGAGTTG 9300
 F F S G R T A K G H K L H Y P M V E Y C
 9301 TTTCTTTTCGGGTCGAACAGCAAAAGGTACAAATTACATTACCCAATGGTGAATATTG 9360
 I P T T S G E D V R D F T K V L K N K F
 9361 TATACCTACACATCTGGGGAAGATGTACGAGACTTCACAAAGGTACTTAAGAACAAGTT 9420
 R S K K Y F A K H P R L G Y L P V Q T V
 9421 CAGGTGGAAGAAGTACTTTGCCAAACACCCTCGACTTGGTTACCTGCCTGTCCAGACAGT 9480
 L E G D N L E T P I T L I S M W P E H Y
 9481 TCTTGAAGGTGACAACCTTAGAGACTCCTATCAGACTCATCAGTATGTGGCCAGAGCACTA 9540
 D P S Q S P Q L F H D D T H S R I E Q Y
 9541 TGACCCCTCACAATCTCCTCAACTGTTTTCATGATGACACCCATTCAAGAATAGAACAATA 9600
 A T R L A Q M E R T N G S F L T D S S S
 9601 TGCCACACGACTGGCCCAGATGGAAAGGACTAATGGGTCTTTTCTCACTGATAGCAGCTC 9660
 T T G S V E D E H A L I Q Q Y C Q T L G
 9661 CACCACAGGAAGTGTGGAAGACGAGCAGCCCTCATCCAGCAGTATTGCCAAACACTCGG 9720
 G E S P V S Q P Q S P A Q I L K S V E R
 9721 AGGAGAGTCCCCAGTGAGCCAGCCGAGAGCCAGCTCAGATCCTGAAGTCAGTAGAGAG 9780
 E E R G E L E R I I A D L E E E Q R N L
 9781 GGAAGAAGCTGGAGAAGTGGAGAGGATCATTGCTGACCTGGAGGAAGAACAAGAAATCT 9840
 Q V E Y E Q L K D Q H L R R G L P V G S
 9841 ACAGGTGGAGTATGAGCAGCTGAAGGACCAGCACCTCCGAAGGGGGCTCCCTGTCGGTTC 9900
 P P E S I I S P H H T S E D S E L I A E
 9901 ACCGCCAGAGTCGATTATATCTCCCCATCACACGCTCTGAGGATTCAGAACTTATAGCAGA 9960
 A K L L R Q H K G R L E A R M Q I L E D
 9961 AGCAAACTCCTCAGGCAGCACAAAGGTGCGCTGGAGGCTAGGATGCAGATTTTAGAAGA 10020

Figure 9 Continued

	H N K Q L E S Q L H R L R Q L L E Q P E	
10021	TCACAATAAACAGCTGGAGTCTCAGCTCCACCGCCTCCGACAGCTGCTGGAGCAGCCTGA	10080
	S D S R I N G V S P W A S P Q H S A L S	
10081	ATCTGATTCCCGAATCAATGGTGTTCCTCCTCAGCATTCTGCACTGAG	10140
	Y S L D P D A S G P Q F H Q A A G E D L	
10141	CTACTCGCTTGATCCAGATGCCTCCGGCCACAGTTCCACCAGGCAGCGGGAGAGGACCT	10200
	L A P P H D T S T D L T E V M E Q I H S	
10201	GCTGGCCCCACCGCAGCACCAGCACGGATCTCACGGAGGTCATGGAGCAGATTCAAG	10260
	T F P S C C P N V P S R P Q A M *	
10261	CACGTTTCCATCTTGCTGCCCAAATGTTCCAGCAGGCCACAGGCAATGTAATCACTAGT	10320

Figure 10

human	MAKYGEHEASPDNGQNEFSUIJKSRSD	EHNDVOKKFTFKHINARFSKS	GKPPINDMF	IDLKDGKRLDL	LLEGLTG	75
mouse	MAKYGEHEASPDNGQNEFSUIJKSRSD	EHNDVOKKFTFKHINARFSKS	GKPPINDMF	IDLKDGKRLDL	LLEGLTG	75
rat	MAKYGHLEASPDNGQNEFSUIJKSRSD	EHNDVOKKFTFKHINARFSKS	GKPPINDMF	IDLKDGKRLDL	LLEGLTG	75
human	TSLPKERGSTRVHALNNVNRVLOVLHONNV	ELVNI	GGTDIVDGNHKLKTLGLLH	HSIILHWOVKDV	VHSDLOOT	150
mouse	TSLPKERGSTRVHALNNVNRVLOVLHONNV	ELVNI	GGTDIVDGNHKLKTLGLLH	HSIILHWOVKDV	VHSDLOOT	150
rat	TSLPKERGSTRVHALNNVNRVLOVLHONNV	ELVNI	GGTDIVDGNHKLKTLGLLH	HSIILHWOVKDV	VHSDLOOT	150
human	NSEKILLSKVROTTIRPYSOVMVNLNFTT	TSHTDGLAFNAVLRHRHKPPDL	FSMDKIVVKHSP	IERLEHAFSKA	QIYTLGLE	225
mouse	NSEKILLSKVROTTIRPYSOVMVNLNFTT	TSHTDGLAFNAVLRHRHKPPDL	FSMDKIVVKHSP	IERLEHAFSKA	QIYTLGLE	225
rat	NSEKILLSKVROTTIRPYSOVMVNLNFTT	TSHTDGLAFNAVLRHRHKPPDL	FSMDKIVVKHSP	IERLEHAFSKA	QIYTLGLE	225
human	KLDPEDVAVRILPDKKSIIMHLYTSLF	EVLPOOVTTDAIREVETL	PRKYKKCECEEE	IAINIQSITAP	EEME	300
mouse	KLDPEDVAVRILPDKKSIIMHLYTSLF	EVLPOOVTTDAIREVETL	PRKYKKCECEEE	IAINIQSITAP	EEME	300
rat	KLDPEDVAVRILPDKKSIIMHLYTSLF	EVLPOOVTTDAIREVETL	PRKYKKCECEEE	IAINIQSITAP	EEME	300

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 C12N15/79 C07K14/47 A61K31/70 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC.

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol. 92, April 1995, pages 3697-3701, XP002031008 BLAKE, D.J., ET AL. : "G-UTROPHIN, THE AUTOSOMAL HOMOLOGUE OF DYSTROPHIN Dp116, IS EXPRESSED IN SENSORY GANGLIA AND BRAIN" see the whole document & EMBL SEQUENCE DATA LIBRARY, 1 May 1995, HEIDELBERG, GERMANY, BLAKE, D.J., ET AL. : "G-UTROPHIN, THE AUTOSOMAL HOMOLOGUE OF DYSTROPHIN Dp 116, IS EXPRESSED IN SENSORY GANGLIA" ACCESSION No. x83506</p> <p style="text-align: center;">--- -/-</p>	3,7,8, 11,17,24

☒ Further documents are listed in the continuation of box C.☐ Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
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"&" document member of the same patent family

Date of the actual completion of the international search

16 May 1997

Date of mailing of the international search report

27.05.97

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+ 31-70) 340-3016

Authorized officer

Holtorf, S

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NATURE, vol. 339, 4 May 1989, pages 55-58, XP002031004 LOVE, D.R., ET AL. : "AN AUTOSOMAL TRANSCRIPT IN SKELETAL MUSCLE WITH HOMOLOGY TO DYSTROPHIN" see the whole document & EMBL SEQUENCE DATA LIBRARY, 6 November 1992, HEIDELBERG, GERMANY, TINSLEY, J.M., ET AL. : "PRIMARY STRUCTURE OF DYSTROPHIN RELATED PROTEIN" ACCESSION No. X69086 ---	3,6,8, 11,17
X	NATURE, vol. 360, 10 December 1992, pages 591-593, XP002031005 TINSLEY, J.M., ET AL. : "PRIMARY STRUCTURE OF DYSTROPHIN-RELATED PROTEIN" cited in the application see the whole document ---	7,8,11, 17
X	HUMAN MOLECULAR GENETICS, vol. 4, no. 8, August 1995, pages 1251-1258, XP002031006 PHELPS, S.F., ET AL. : "EXPRESSION OF FULL-LENGTH AND TRUNCATED DYSTROPHIN MINI-GENES IN TRANSGENIC mdx MICE" see the whole document ---	3,7-9, 11-21, 24,27,29
P,X	NATURE, vol. 384, 28 November 1996, pages 349-353, XP002031010 TINSLEY, J.M., ET AL. : "AMELIORATION OF THE DYSTROPHIC PHENOTYPE OF mdx MICE USING A TRUNCATED UTROPHIN TRANSGENE" see the whole document ---	1,2,4, 8-21,24, 25,27-29
A	NEUROMUSCULAR DISORDERS, vol. 3, no. 1, January 1993, pages 5-21, XP000673607 LOVE, D.R., ET AL. : "DYSTROPHIN AND DYSTROPHIN-RELATED PROTEINS: A REVIEW OF PROTEIN AND RNA STUDIES" see the whole document ---	1-30
A	CELL, vol. 53, 22 April 1988, pages 219-228, XP002031011 KÖNIG, M., ET AL. : "THE COMPLETE SEQUENCE OF DYSTROPHIN PREDICTS A ROD-SHAPED CYTOSKELETAL PROTEIN" see the whole document ---	1-30

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	NEUROMUSCULAR DISORDERS, vol. 3, no. 5/6, 1993, pages 537-539, XP000578145 TINSLEY, J.M., ET AL. : "UTROPHIN: A POTENTIAL REPLACEMENT FOR DYSTROPHIN ?" see the whole document -----	1-30
A	FEBS LETTERS, vol. 358, 1995, pages 262-266, XP000486346 THI MAN, N., ET AL. : "FULL-LENGTH AND SHORT FORMS OF UTROPHIN, THE DYSTROPHIN-RELATED PROTEIN" see the whole document -----	1-30

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 29, 17, 18, 20-23, 27, 28
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 29 and 17, 18, 20-23, 27, 28 as far as in vivo methods are concerned are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

